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(54) Title: METHODS FOR TREATMENT OF CANCER USING IRINOTECAN BASED ON UGT1A1

(57) Abstract: The present invention relates to the use of irinotecan or a derivative thereof for the preparation of a pharmaceutical composition for treating colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a patient having a genotype with a variant allele which comprises a polynucleotide in accordance with the present invention. Preferably, a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in a altered expression of a variant allele compared to the corresponding wild type allele or an altered activity of the polypeptide encoded by the variant allele compared to the polypeptide encoded by the corresponding wild type allele. Finally, the present invention relates to a method for selecting a suitable therapy for a subject suffering from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer.



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## METHODS FOR TREATMENT OF CANCER USING IRINOTECAN BASED ON UGT1A1

The present invention relates to the use of camptothecin drugs, such as irinotecan (CPT-11) or a derivative thereof for the preparation of a pharmaceutical composition for treating colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a patient having a genotype with a variant allele which comprises a polynucleotide in accordance with the present invention. Preferably, a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered expression of the variant allele compared to the corresponding wild type allele or an altered activity of the polypeptide encoded by the variant allele compared to the polypeptide encoded by the corresponding wild type allele. Finally, the present invention relates to a method for selecting a suitable therapy for a subject suffering from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer or pancreatic cancer.

Irinotecan is a semisynthetic analog of the cytotoxic alkaloid camptothecin (CPT), which is obtained from the oriental tree, *Camptotheca acuminata*. Camptothecins demonstrate anti-neoplastic activities by inhibiting specifically with the enzyme topoisomerase I which relieves torsional strain in DNA by inducing reversible single-strand breaks [D'Arpa, *et al.*, 1989, *Biochim Biophys Acta* 989:163-77, Horwitz, *et al.*, 1973, *Cancer Res* 33:2834-6]. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks [Kawato, *et al.*, 1991, *Cancer Res* 51:4187-91]. Irinotecan serves as a water-soluble prodrug of the lipophilic metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) which is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and

the dipiperidino side chain [Tsuji, *et al.*, 1991, J Pharmacobiodyn 14:341-9]. Carboxylesterase-2 is the primary enzyme involved in this hydrolysis at pharmacological concentrations [Humerickhouse, *et al.*, 2000, Cancer Res 60:1189-92]. Topoisomerase inhibition and irinotecan-related single strand breaks are caused primarily by SN-38 [Kawato, *et al.*, 1991, Cancer Res 51:4187-91]. Administration of irinotecan has resulted in antitumor activity in mice bearing cancers of rodent origin and in human carcinoma xenografts of various histological types [Furuta, *et al.*, 1988, Gan To Kagaku Ryoho 15:2757-60, Giovanella, *et al.*, 1989, Science 246:1046-8, Giovanella, *et al.*, 1991, Cancer Res 51:3052-5, Hawkins, 1992, Oncology (Huntingt) 6:17-23, Kunimoto, *et al.*, 1987, Cancer Res 47:5944-7].

Irinotecan is also oxidized by CYP3A4 and CYP3A5 [Haaz, *et al.*, 1998, Drug Metab Dispos 26:769-74, Kuhn, 1998, Oncology (Huntingt) 12:39-42, Santos, *et al.*, 2000, Clin Cancer Res 6:2012-20, Rivory, *et al.*, 1996, Cancer Res 56:3689-94]. The major elimination pathway of SN-38 is conjugation with glucuronic acid to form the corresponding glucuronide (SN-38G) [Atsumi, *et al.*, 1991, Xenobiotica 21:1159-69]. SN-38G is reported to be deconjugated by the intestinal microflora to form SN-38 [Kaneda, *et al.*, 1990, Cancer Res 50:1715-20]. Glucuronidation of SN-38 is mediated by UGT1A1 and UGT1A7 [Iyer, *et al.*, 1998, J Clin Invest 101:847-54, Ciotti, *et al.*, 1999, Biochem Biophys Res Commun 260:199-202]. Mass balance studies have demonstrated that 64% of the total dose is excreted in the feces, confirming the important role of biliary excretion [Slatter, *et al.*, 2000, Drug Metab Dispos 28:423-33]. Studies suggest that the multidrug resistance protein 1 (MRP1) is a major transporter of irinotecan and its metabolites [Kuhn, 1998, Oncology (Huntingt) 12:39-42, Chen, *et al.*, 1999, Mol Pharmacol 55:921-8, Chu, *et al.*, 1997, Cancer Res 57:1934-8, Chu, *et al.*, 1997, J Pharmacol Exp Ther 281:304-14] and facilitate their biliary excretion, where they cause side effects, although P-glycoprotein also participates in irinotecan excretion [Chu, *et al.*, 1998, Cancer Res 58:5137-43, Chu, *et al.*, 1999, Drug Metab Dispos 27:440-1, Chu, *et al.*, 1999, J Pharmacol Exp Ther 288:735-41, Mattern, *et al.*, 1993, Oncol Res 5:467-74, Hoki, *et al.*, 1997, Cancer Chemother Pharmacol 40:433-8, Sugiyama, *et al.*, 1998, Cancer Chemother Pharmacol 42:S44-9].

Cellular resistance to camptothecins and thus, therapeutic response of irinotecan has been related to intracellular carboxylesterase activity and cleavage activity of topoisomerase I [van Ark-Otte, *et al.*, 1998, Br J Cancer 77:2171-6, Guichard, *et al.*, 1999, Br J Cancer 80:364-70].

The use of such camptothecin drugs, e.g. irinotecan, is limited by clearly dose-dependent myelosuppression and gastrointestinal toxicities, including nausea, vomiting, abdominal pain, and diarrhea which side effects can prove fatal. The major dose-limiting toxicity of irinotecan therapy is diarrhea, which occurs in up to 88% of patients and which depends on intestinal SN-38 accumulation [van Ark-Otte, *et al.*, 1998, Br J Cancer 77:2171-6, Guichard, *et al.*, 1999, Br J Cancer 80:364-70, Araki, *et al.*, 1993, Jpn J Cancer Res 84:697-702] secondary to the biliary excretion of SN-38, the extent of which is determined by SN-38 glucuronidation [Gupta, *et al.*, 1994, Cancer Res 54:3723-5, Gupta, *et al.*, 1997, J Clin Oncol 15:1502-10]. Myelosuppression has been correlated with the area under the concentration-time curve of both irinotecan and SN-38 [Sasaki, *et al.*, 1995, Jpn J Cancer Res 86:101-10].

Despite the approval of irinotecan for patients with metastatic colorectal cancer refractory to 5-fluorouracil therapy in 1997, the therapeutic benefit remains questionable. Recently two large clinical trials on colorectal cancer involving more than 2000 patients had to be canceled by the National Institute of Cancer (NCI) due to an almost 3-times increase of irinotecan toxicity-related mortality within the first 60 days of treatment. Causes of death were diarrhea- and vomiting-related dehydration and neutropenia-related sepsis [2001, arznei-telegramm 32:58]. Although irinotecan was proven to be effective against the cancer itself, not all patients could benefit from longterm survival due to short term toxicity. Thus, it is highly desirable to identify those patients who will most likely suffer from irinotecan toxicity.

Currently, patients are treated according to most treatment schedules with a standard dose of initially 60 to 125 mg/m<sup>2</sup> irinotecan in combination with other anti-neoplastic drugs administered several courses of 3 to 4 weekly dosings, and subsequent doses are adjusted in 25 to 50 mg/m<sup>2</sup> increments based upon individual patient tolerance to treatment. Treatment may be delayed 1 to 2 weeks to

allow for recovery from irinotecan-related toxicity and if the patient has not recovered, therapy has to be discontinued. Provided intolerable toxicity does not develop, treatment with additional courses are continued indefinitely as long as the patient continues to experience clinical benefit. Response rates varies depending from tumor type from less than 10 % to almost 90 %. However, it takes at least 6 to 8 weeks to evaluate therapeutic response and to consider alternatives. Thus, finding the right dosage for the patient is tedious, time-consuming and takes the risk of lifethreatening adverse effects. Patients might be unnecessarily put to this risk who do not benefit from treatment and additionally, worthwhile time is wasted before these patients receive their suitable treatment.

Furthermore, as observed for many chemotherapeutic agents, the risk to develop cellular resistances against therapy is increased upon suboptimal exposure of cells to chemotherapeutic agents, such as irinotecan.

Pharmacokinetic modulation with inhibitors of biliary excretion (*e. g.*, MRP and P-glycoprotein) and inducers of UGT1A1 have been suggested as a tool to reduce camptothecin-related toxicity [Gupta, *et al.*, 1996, Cancer Res 56:1309-14, Gupta, *et al.*, 1997, Cancer Chemother Pharmacol 39:440-4]. Although preliminary data of a clinical study of irinotecan in combination with cyclosporine A, and phenobarbital show some promising results in respect to limit camptothecin-related diarrhea [Ratain, 2000, Clin Cancer Res 6:3393-4], cotreatment with drugs such as cyclosporine A, and phenobarbital takes the additional risk of adverse events and drug interactions.

Large interpatient variability exist for both SN-38 and SN-38G pharmacokinetics [Canal, *et al.*, 1996, J Clin Oncol 14:2688-95], which is likely to be due to interpatient differences in the metabolism pathways of irinotecan [Rivory, *et al.*, 1997, Clin Cancer Res 3:1261-6]. Furthermore, severe irinotecan toxicity has been reported in patients with Gilbert syndrome [Wasserman, *et al.*, 1997, Ann Oncol 8:1049-51]. Consequently, a genetic predisposition to the metabolism of irinotecan, that patients with low UGT1A1 activity are at increased risk for irinotecan toxicity has been suggested [Iyer, *et al.*, 1998, J Clin Invest 101:847-54, Ando, *et al.*, 1998, Ann Oncol 9:845-7]. A common polymorphism in the UGT1A1 promoter [Monaghan, *et al.*, 1996, Lancet 347:578-81] has been correlated with *in vitro*

glucuronidation of SN-38 [Iyer, *et al.*, 1999, Clin Pharmacol Ther 65:576-82], and its possible clinical use has been suggested from a case control study [Ando, *et al.*, 2000, Cancer Res 60:6921-6]. However, irinotecan-related toxicity was predicted by UGT1A1 genotype only in the minority of affected patients (< 15 %).

In conclusion, it would be highly desirable to significantly improve therapeutic efficacy and safety of camptothecin-based therapies and to avoid therapy-caused fatalities, to avoid unnecessary development of resistances, and to reduce adverse events- and therapeutic delay-related hospitalization costs. However, no accepted mechanism for reducing irinotecan toxicity or to improve therapeutic efficacy are currently available.

Thus, the technical problem underlying the present invention is to provide improved means and methods for the efficient treatment of colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer, whereby the aforementioned undesirable side effects are to be avoided.

The technical problem underlying the present invention is solved by the embodiments characterized in the claims.

Accordingly, the present invention relates to the use of irinotecan or a derivative thereof for the preparation of a pharmaceutical composition for treating colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a subject having a genome with a variant allele which comprises a polynucleotide selected from the group consisting of:

- (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 001, 002, 005, 006, 009, 010, 013, 014, 017, 018, 021, 022, 025, 026, 029, 030, 033, 034, 037, 038, 041, 042, 045, 046, 049, 050, 053, 054, 057, 058, 061, 062, 065, 066, 069, 070, 073, 074, 077, 078, 081, 082, 085, 086, 089, 090, 093, 094, 097, 098, 101, 102, 105, 106, 109, 110, 113, 114, 129, 130, 133 and/or 134;

- (b) a polynucleotide encoding a polypeptide having the amino acid sequence of any one of SEQ ID NOs: 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596 and/or 598;
- (c) a polynucleotide capable of hybridizing to a Uridine Diphosphate Glycosyltransferase1 Member A1 (UGT1A1) gene, wherein said polynucleotide is having at a position corresponding to positions 59, 160, 226, 539, 544, 640, 701, 841, 855, 890, 938, 1006, 1007, 1020, 1084, 1085, 1114, 1117, 1139, 1158, 1175 to 1176, 1216, 1297, 1324, 1471, 1478, 372 to 373, 523 to 525, and/or 892 to 905 of the UGT1A1 gene (Accession No. GI:8850235), a substitution or deletion of at least one nucleotide or at a position corresponding to positions 470/471, and/or 1222/1223 of the UGT1A1 gene (Accession No. GI:8850235) a insertion of at least one nucleotide;
- (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having at a position corresponding to position 226, 539, 701, 855, 938, 1020, and/or 1117 of the UGT1A1 gene (Accession No: GI:8850235) an A, at a position corresponding to position 160, 640, 890, 1006, 1084, 1139, 1176, 1324, and/or 1478 of the UGT1A1 gene (Accession No: GI: 8850235) a T, at a position corresponding to position 544, 841, and/or 1216 of the UGT1A1 gene (Accession No: GI: 8850235) a C, at a position corresponding to position 59, 1007, 1085, 1114, 1158, 1175, 1297, and/or 1471 of the UGT1A1 gene (Accession No: GI:181303) a G, and/or at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TTC, at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TACATTAATGCTTC, at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a T, and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a G;

- (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution of Leu to Arg at a position corresponding to position 15 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 71 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Leu to Gln at a position corresponding to position 175 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Cys to Arg at a position corresponding to position 177 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Trp at a position corresponding to position 209 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Gln at a position corresponding to position 229 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 276 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Val at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Trp at a position corresponding to position 293 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Glu at a position corresponding to position 308 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding to position 331 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding to position 357 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Gly at a position corresponding to position 367 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Thr at a position corresponding to position 368 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Arg at a position corresponding to position 387 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a position corresponding to position 375 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Arg at a position corresponding to position 381 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Pro at a position corresponding to position 401 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Lys to Glu at a position corresponding to position 428 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Asp at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a

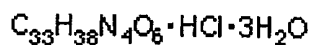
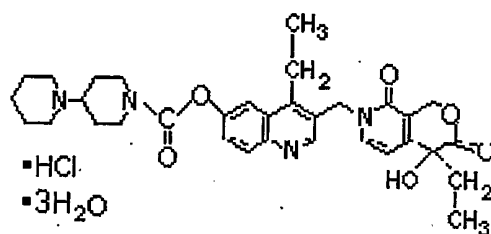


position corresponding to position 488 of the UGT1A1 polypeptide (Accession No: G8850236);

- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide is having at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, whereby in said polypeptide one or more amino acids following amino acid Asp at a position corresponding to position 119 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a T, whereby in said polypeptide one or more amino acids following amino acid Pro at a position corresponding to position 152 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TTC, whereby in said polypeptide one or more amino acids following amino acid Thr at a position corresponding to position 168 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TACATTAATGCTTC, whereby in said polypeptide one or more amino acids following amino acid Ala at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a G, whereby in said polypeptide one or more amino acids following amino acid Lys at a position corresponding to position 402 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted; and
- (g) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide comprises an amino acid substitution of Gln to a stop codon at a position corresponding to position 49 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Cys to a stop codon at a position corresponding to position 280 of the UGT1A1 gene

(Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 331 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Trp to a stop codon at a position corresponding to position 335 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 357 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Lys to a stop codon at a position corresponding to position 437 of the UGT1A1 gene (Accession No: G8850236).

The term "irinotecan or a derivative thereof" as used in accordance with the present invention preferably refers to a substance which is characterized by the general structural formula



further described in US patents US05106742, US05340817, US05364858, US05401747, US05468754, US05559235 and US05663177. Moreover, also comprised by the term "irinotecan or a derivative thereof" are analogues and derivatives of camptothecin. The types and ranges of camptothecin analogues available are well known to those of skill in the art and described in numerous texts, e.g. [Hawkins, 1992, Oncology (Huntingt) 6:17-23, Burris, *et al.*, 1994, Hematol Oncol Clin North Am 8:333-55, Slichenmyer, *et al.*, 1993, J Natl Cancer Inst 85:271-91, Slichenmyer, *et al.*, 1994, Cancer Chemother Pharmacol 34:S53-7]. Specific examples of active camptothecin analogues are hexacyclic camptothecin

analogues, 9-nitro-camptothecin, camptothecin analogues with 20S configuration with 9- or 10-substituted amino, halogen, or hydroxyl groups, seven-substituted water-soluble camptothecins, 9-substituted camptothecins, E-ring-modified camptothecins such as (RS)-20-deoxyamino-7-ethyl-10-methoxycamptothecin, and 10-substituted camptothecin analogues [Emerson, *et al.*, 1995, Cancer Res 55:603-9, Ejima, *et al.*, 1992, Chem Pharm Bull (Tokyo) 40:683-8, Sugimori, *et al.*, 1994, J Med Chem 37:3033-9, Wall, *et al.*, 1993, J Med Chem 36:2689-700, Wani, *et al.*, 1980, J Med Chem 23:554-60, Kingsbury, *et al.*, 1991, J Med Chem 34:98-107]. Various other camptothecin analogues with similar therapeutic activity are described [Hawkins, 1992, Oncology (Huntingt) 6:17-23, Burris and Fields, 1994, Hematol Oncol Clin North Am 8:333-55, Slichenmyer, *et al.*, 1993, J Natl Cancer Inst 85:271-91, Slichenmyer, *et al.*, 1994, Cancer Chemother Pharmacol 34:S53-7]. Suitable methods for synthesizing camptothecin analogues are described [Emerson, *et al.*, 1995, Cancer Res 55:603-9, Ejima, *et al.*, 1992, Chem Pharm Bull (Tokyo) 40:683-8, Sugimori, *et al.*, 1994, J Med Chem 37:3033-9, Wall, *et al.*, 1993, J Med Chem 36:2689-700, Wani, *et al.*, 1980, J Med Chem 23:554-60, Kingsbury, *et al.*, 1991, J Med Chem 34:98-107, Sugasawa, *et al.*, 1976, J Med Chem 19:675-9].

Said substances are known to be therapeutically useful as described, e.g., in colorectal cancer, non-small cell and small cell lung cancer, oesophageal cancer, renal cell carcinoma, ovarian cancer, breast cancer, pancreatic cancer, squamous cell cancer, leukemias and lymphomas [Kawato, *et al.*, 1991, Cancer Res 51:4187-91, Furuta, *et al.*, 1988, Gan To Kagaku Ryoho 15:2757-60, Hawkins, 1992, Oncology (Huntingt) 6:17-23, Slichenmyer, *et al.*, 1993, J Natl Cancer Inst 85:271-91, Slichenmyer, *et al.*, 1994, Cancer Chemother Pharmacol 34:S53-7, Tsuruo, *et al.*, 1988, Cancer Chemother Pharmacol 21:71-4, Wiseman, *et al.*, 1996, Drugs 52:606-23, Gottlieb, *et al.*, 1970, Cancer Chemother Rep 54:461-70, Negoro, *et al.*, 1991, J Natl Cancer Inst 83:1164-8, Rowinsky, *et al.*, 1994, Cancer Res 54:427-36]. Also encompassed by the use of the present invention are derivatives of those substances which are obtainable by way of any chemical modification, wherein said derivatives are equally well therapeutically suited for the use of the present invention. To determine whether a derivative of the substances of the invention is equally well therapeutically suited for the use of the invention biological assays well

known in the art can be performed. Such assays are described, e.g., in [Kawato, *et al.*, 1991, *Cancer Res* 51:4187-91, Furuta, *et al.*, 1988, *Gan To Kagaku Ryoho* 15:2757-60, Giovanella, *et al.*, 1989, *Science* 246:1046-8, Giovanella, *et al.*, 1991, *Cancer Res* 51:3052-5, Kunimoto, *et al.*, 1987, *Cancer Res* 47:5944-7, Mattern, *et al.*, 1993, *Oncol Res* 5:467-74, Tsuruo, *et al.*, 1988, *Cancer Chemother Pharmacol* 21:71-4, Burris, *et al.*, 1992, *J Natl Cancer Inst* 84:1816-20, Friedman, *et al.*, 1994, *Cancer Chemother Pharmacol* 34:171-4].

It is contemplated that any of the compounds described in the above publications may be used in this invention.

It has been shown that irinotecan is particularly well suited for the treatment of colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer. Thus, most preferably the substance used according to the present invention is irinotecan.

The term "pharmaceutical composition" as used herein comprises the substances of the present invention and optionally one or more pharmaceutically acceptable carrier. The substances of the present invention may be formulated as pharmaceutically acceptable salts. Acceptable salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The pharmaceutical compositions can be conveniently administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The substances may be administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid

carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax. The substance according to the present invention can be administered in various manners to achieve the desired effect. Said substance can be administered either alone or in the formulated as pharmaceutical preparations to the subject being treated either orally, topically, parenterally or by inhalation. Moreover, the substance can be administered in combination with other substances either in a common pharmaceutical composition or as separated pharmaceutical compositions.

The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. A therapeutically effective dose refers to that amount of the substance according to the invention which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED<sub>50</sub> (the dose therapeutically effective in 50% of the population) and LD<sub>50</sub> (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD<sub>50</sub>/ED<sub>50</sub>.

The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment.

A typical dose can be, for example, in the range of 5 to 100 mg however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the

pharmaceutical composition should be in the range of 1  $\mu\text{g}$  to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 1  $\mu\text{g}$  to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. However, depending on the subject and the mode of administration, the quantity of substance administration may vary over a wide range to provide from about 1 mg per  $\text{m}^2$  body surface to about 500 mg per  $\text{m}^2$  body surface, usually 20 to 200 mg per  $\text{m}^2$  body surface.

The pharmaceutical compositions and formulations referred to herein are administered at least once in accordance with the use of the present invention. However, the said pharmaceutical compositions and formulations may be administered more than one time, for example once weekly every other week up to a non-limited number of weeks.

Specific formulations of the substance according to the invention are prepared in a manner well known in the pharmaceutical art and usually comprise at least one active substance referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent thereof. For making those formulations the active substance(s) will usually be mixed with a carrier or diluted by a diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. A carrier may be solid, semisolid, gel-based or liquid material which serves as a vehicle, excipient or medium for the active ingredients. Said suitable carriers comprise those mentioned above and others well known in the art, see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania. The formulations can be adopted to the mode of administration comprising the forms of tablets, capsules, suppositories, solutions, suspensions or the like.

The dosing recommendations will be indicated in product labeling by allowing the prescriber to anticipate dose adjustments depending on the considered patient group, with information that avoids prescribing the wrong drug to the wrong patients at the wrong dose.

The term "treating" means alleviation of the diseases symptoms, i.e., regression of symptoms or inhibited progression of such symptoms, in subjects or disease

populations which have been treated. Said alleviation of the diseases can be monitored by the degree of the clinical symptoms (e.g., tumor size) accompanied with the disease. While the invention may not be effective in 100% of patients treated, it is effective in treating a statistically significant ( $p$  value less than 0.05) number of patients. Whether said number of subjects is significant can be determined by statistical tests such as the Student's  $t$ -test, the  $\chi^2$ -test, the U-test according to Mann and Whitney, the Kruskal-Wallis-test (H-Test), Jonckheere-Terpstra-test or the Wilcoxon-test.

The present invention also encompasses all embodiments described in connection with pharmaceutical compositions in US patents US05106742, US05340817, US05364858, US05401747, US05468754, US05559235 and US05663177.

The terms "colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer" comprise diseases and dysregulations related to cancer. Preferred diseases encompassed by the use of the present invention are colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer. Said diseases and dysregulations are well known in the art and the accompanied symptoms are described, e.g., in standard text books such as Stedman.

The term "subject" as used in the sense of the present invention comprises animals, preferably those specified herein after, and humans.

The term "variant allele" as used herein refers to a polynucleotide comprising one or more of the polynucleotides described herein below corresponding to a UGT1A1 gene. Each individual subject carries at least two alleles of the UGT1A1 gene, wherein said alleles are distinguishable or identical. In accordance with the use of the present invention a variant allele comprises at least one or more of the polynucleotides specified herein below. Said polynucleotides may have a synergistic influence on the regulation or function of the first variant allele. Preferably, a variant allele in accordance with the use of the present invention comprises at least two of the polynucleotides specified herein.

In the context of the present invention the term "polynucleotides" or "polypeptides" refers to different variants of a polynucleotide or a polypeptide specified in accordance with the uses of the present invention. Said variants comprise a reference or wild type sequence of the polynucleotides or polypeptides specified herein as well as variants which differ therefrom in structure or composition. Reference or wild type sequences for the polynucleotides those GenBank accession numbers referred to above. The differences in structure or composition usually occur by way of nucleotide or amino acid substitution(s), addition(s) and/or deletion(s).

Preferably, said nucleotide substitution(s), addition(s) or deletion(s) referred to in accordance with the use of the present invention result(s) in one or more changes of the corresponding amino acid(s) of the polypeptides. The variant polynucleotides also comprise fragments of said polynucleotides or polypeptides. The polynucleotides or polypeptides as well as the aforementioned fragments thereof are characterized as being associated with a UGT1A1 dysfunction or dysregulation comprising, e.g., insufficient and/or altered drug metabolism.

The present invention also encompasses all embodiments described in connection with polynucleotides in WO9957322, WO0109183 or US5786344.

The term "hybridizing" as used herein refers to polynucleotides which are capable of hybridizing to the above polynucleotides or parts thereof which are associated with a UGT1A1 dysfunction or dysregulation. Thus, said hybridizing polynucleotides are also associated with said dysfunctions and dysregulations. Preferably, said polynucleotides capable of hybridizing to the aforementioned polynucleotides or parts thereof which are associated with UGT1A1 dysfunctions or dysregulations are at least 70%, at least 80%, at least 95% or at least 100% identical to the polynucleotides or parts thereof which are associated with UGT1A1 dysfunctions or dysregulations. Therefore, said polynucleotides may be useful as probes in Northern or Southern Blot analysis of RNA or DNA preparations, respectively, or can be used as oligonucleotide primers in PCR analysis dependent on their respective size. Also comprised in accordance with the use of the invention are hybridizing polynucleotides which are useful for analyzing DNA-Protein interactions via, e.g., electrophoretic mobility shift analysis (EMSA). Preferably, said hybridizing



polynucleotides comprise at least 10, more preferably at least 15 nucleotides in length while a hybridizing polynucleotide to be used as a probe preferably comprises at least 100, more preferably at least 200, or most preferably at least 500 nucleotides in length.

It is well known in the art how to perform hybridization experiments with nucleic acid molecules, i.e. the person skilled in the art knows what hybridization conditions s/he has to use in accordance with the present invention. Such hybridization conditions are referred to in standard text books, such as *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory (1989) N.Y. Preferred in accordance with the use of the present inventions are polynucleotides which are capable of hybridizing to the above polynucleotides or parts thereof which are associated with a UGT1A1 dysfunction or dysregulation under stringent hybridization conditions, i.e. which do not cross hybridize to unrelated polynucleotides such as polynucleotides encoding a polypeptide different from the UGT1A1 polypeptides of the invention.

Moreover, methods for determining whether a subject comprises a polynucleotide referred to herein above are well known in the art. To carry out said methods, it might be necessary to take a sample comprising biological material, such as isolated cells or tissue, from said subject. Further, the methods known in the art could comprise for example, PCR based techniques, RFLP-based techniques, DNA sequencing-based techniques, hybridization techniques, Single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), mismatch cleavage detection, heteroduplex analysis, techniques based on mass spectroscopy, HPLC-based techniques, primer extension-based techniques, and 5'-nuclease assay-based techniques. A preferred and convenient method to be used in order to determine the presence or absence of one or more of the above specified polynucleotides is to isolate blood cells from a subject and to perform a PCR based assay on genomic DNA isolated from those blood cells, whereby the PCR is used to determine whether said polynucleotides specified herein above or parts thereof are present or absent. Said method is described in more detail below and in the Examples.

The term "corresponding", as used herein means that a position is not only determined by the number of the preceding nucleotides and amino acids,

respectively. The position of a given nucleotide or amino acid in accordance with the use of the present invention which may be deleted, substituted or comprise one or more additional nucleotide(s) may vary due to deletions or additional nucleotides or amino acids elsewhere in the gene or the polypeptide. Thus, under a "corresponding position" in accordance with the present invention it is to be understood that nucleotides or amino acids may differ in the indicated number but may still have similar neighboring nucleotides or amino acids. Said nucleotides or amino acids which may be exchanged, deleted or comprise additional nucleotides or amino acids are also comprised by the term "corresponding position". Said nucleotides or amino acids may for instance together with their neighbors form sequences which may be involved in the regulation of gene expression, stability of the corresponding RNA or RNA editing, as well as encode functional domains or motifs of the protein of the invention.

By, e.g., "position 372 to 373" it is meant that said polynucleotide comprises one or more deleted nucleotides which are deleted between positions 372 and position 373 of the corresponding wild type version of said polynucleotide. The same applies mutatis mutandis to all other position numbers referred to in the above embodiment which are drafted in the same format.

By, e.g., "position 470/471" it is meant that said polynucleotide comprises one or more additional nucleotide(s) which are inserted between positions 470 and position 471 of the corresponding wild type version of said polynucleotide. The same applies mutatis mutandis to all other position numbers referred to in the above embodiment which are drafted in the same format, i.e. two consecutive position numbers separated by a slash (/).

In accordance with the present invention, the mode and population distribution of genetic variations in the UGT1A1 gene - the different alleles of the UGT1A1 gene - have been analyzed by sequence analysis of relevant regions of the human said gene from many different individuals. It is a well known fact that genomic DNA of individuals, which harbor the individual genetic makeup of all genes, including the UGT1A1 gene, can easily be purified from individual blood samples. These individual DNA samples are then used for the analysis of the sequence composition of the alleles of the UGT1A1 gene that are present in the individual which provided

the blood sample. The sequence analysis was carried out by PCR amplification of relevant regions of said genes, subsequent purification of the PCR products, followed by automated DNA sequencing with established methods (e.g. ABI dyeterminator cycle sequencing).

One important parameter that has to be considered in the attempt to determine the individual genotypes and identify novel variants of the UGT1A1 gene by direct DNA-sequencing of PCR-products from human blood genomic DNA is the fact that each human harbors (usually, with very few abnormal exceptions) two gene copies of each autosomal gene (diploidy). Because of that, great care has to be taken in the evaluation of the sequences to be able to identify unambiguously not only homozygous sequence variations but also heterozygous variations. The details of the different steps in the identification and characterization of the polymorphisms in the UGT1A1 gene (homozygous and heterozygous) are described in the Examples below.

Over the past 20 years, genetic heterogeneity has been increasingly recognized as a significant source of variation in drug response. Many scientific communications (Meyer, *Ann. Rev. Pharmacol. Toxicol.* 37 (1997), 269-296 and West, *J. Clin. Pharmacol.* 37 (1997), 635-648) have clearly shown that some drugs work better in some patients than in others or may even be highly toxic and that such variations in patients' responses to drugs can be correlated to a molecular basis. This "pharmacogenomic" concept spots correlations between responses to drugs and genetic profiles of patient's (Marshall, *Nature Biotechnology*, 15 (1997), 954-957; Marshall, *Nature Biotechnology*, 15 (1997), 1249-1252). In this context of population variability with regard to drug therapy, pharmacogenomics has been proposed as a tool useful in the identification and selection of patients which can respond to a particular drug without side effects. This identification/selection can be based upon molecular diagnosis of genetic polymorphisms by genotyping DNA from leukocytes in the blood of a patient, for example, and characterization of disease (Bertz, *Clin. Pharmacokinet.* 32 (1997), 210-256; Engel, *J. Chromatogra. B. Biomed. Appl.* 678 (1996), 93-103). For the founders of health care, such as health maintenance organizations in the US and government public health services in many European countries, this pharmacogenomics approach can represent a way of both improving health care and reducing costs related to health care caused by

the development of unnecessary drugs, by ineffective drugs and by side effects due to drug administration.

The mutations in the variant genes of the invention sometimes result in amino acid deletion(s), insertion(s) and in particular in substitution(s) either alone or in combination. It is of course also possible to genetically engineer such mutations in wild type genes or other mutant forms. Methods for introducing such modifications in the DNA sequence of said genes are well known to the person skilled in the art; see, e.g., Sambrook, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory (1989) N.Y.

For the investigation of the nature of the alterations in the amino acid sequence of the polypeptides of the invention may be used such as BRASMOL that are obtainable from the Internet. Furthermore, folding simulations and computer redesign of structural motifs can be performed using other appropriate computer programs (Olszewski, *Proteins* 25 (1996), 286-299; Hoffman, *Comput. Appl. Biosci.* 11 (1995), 675-679). Computers can be used for the conformational and energetic analysis of detailed protein models (Monge, *J. Mol. Biol.* 247 (1995), 995-1012; Renouf, *Adv. Exp. Med. Biol.* 376 (1995), 37-45). These analysis can be used for the identification of the influence of a particular mutation on metabolism, binding, inhibition, mediating of therapeutic action and/or transport of drugs. Moreover, based on the knowledge of the altered structure of the polypeptides which are encoded by the polynucleotides specified in the use of the present invention derivatives of the substances referred to above can be designed and synthesized which can be more efficiently metabolized, modified, transported, eliminated, and/or binded. Thereby, drugs or pro-drugs can be designed on the basis of the substances referred to herein which are more efficient in therapy of colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a subject having a genotype characterized by the presence of one or more polynucleotides of the invention.

Usually, said amino acid deletion, addition or substitution in the amino acid sequence of the protein encoded by the polynucleotide referred to in accordance with the use of the present invention is due to one or more nucleotide substitution, insertion or deletion, or any combinations thereof. Preferably said nucleotide

substitution, insertion or deletion may result in an amino acid substitution of Gln to a stop codon at a position corresponding to position 49 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Cys to a stop codon at a position corresponding to position 280 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 331 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Trp to a stop codon at a position corresponding to position 335 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 357 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Lys to a stop codon at a position corresponding to position 437 of the UGT1A1 gene (Accession No: G8850236). The polypeptides encoded by the polynucleotides referred to in accordance with the use described herein have altered biological properties due to the mutations referred to in accordance with the present invention. Examples for said altered properties are stability of the polypeptides or amount of the polypeptides which may be effected resulting in, e.g. an altered drug metabolism or an altered transport of drugs or an altered substrate specificity or an altered catalytic activity characterized by, e.g. insufficiencies in drug metabolism or a complete loss of the capability to metabolize drugs or an enhanced capacity to metabolize drugs or an altered transport activity characterized by, e.g., insufficiencies in drug transport or a complete loss of the capability of transporting drugs or an altered substrate binding characterized by, e.g. an altered drug action or an altered inhibition or induction of transport or an altered binding to receptors or other target molecules characterized by, e.g. an altered activation of signal transduction pathways or an altered protein or enzyme function. These altered properties result in an impaired pharmacological response to the substances referred to above of the subject to be treated in accordance with the use of the present invention. Moreover, due to said altered properties of the polypeptides encoded by the variant alleles specified herein the substances may be chemically modified in a way resulting in derivatives of the substances which are harmful or toxic for the subject or which cause undesirable side effects.

The mutations in the UGT1A1 gene detected in accordance with the present invention are listed in Tables 1 and 2. As is evident to the person skilled in the art,

the genetic knowledge of the polynucleotides specified herein above can be used to exactly and reliably characterize the genotype of a patient.

Advantageously, therapeutical measures which are based on irinotecan or a derivative thereof can be more efficiently applied when taking into consideration said genetic knowledge. Undesirable side effects of said substances can be avoided and an effective but not harmful dosage can be calculated individually due the knowledge of the genetic makeup of the subject. Moreover in accordance with the foregoing, in cases where a given drug causes an unusual effect, a suitable individual therapy can be designed based on the knowledge of the individual genetic makeup of a subject. This tailored therapy will also be suitable to avoid the occurrence of therapy resistances. Said resistances are one major problem in cancer chemotherapy with various chemotherapeutic agents, this fact being well known in the art. The use of the present invention, therefore, provides an improvement of the therapeutic applications which are based on the known therapeutically desirable effects of the substances referred to herein above since it is possible to individually treat the subject with an appropriate dosage and/or an appropriate derivative of said substances. Thereby, undesirable, harmful or toxic effects are efficiently avoided. Furthermore, the use of the present invention provides an improvement of the therapeutic applications which are based on the known therapeutically desirable effects of the substances referred to herein above since it is possible to identify those subject prior to onset of drug therapy and treat only those subjects with an appropriate dosage and/or an appropriate derivative of said substances who are most likely to benefit from therapy with said substances. Thereby, the unnecessary and potentially harmful treatment of those subjects who do not respond to the treatment with said substances (nonresponders), as well as the development of drug resistances due to suboptimal drug dosing. can be avoided.

In accordance with the present invention it has been surprisingly found that a variant allele corresponding to the UGT1A1 gene which alters the pharmacological response of said subject to the administration of irinotecan or a derivative thereof. As has been found in accordance with the present invention, the pharmacokinetics of a drug which is based on irinotecan or a derivative thereof and the pharmacological response of a subject is mainly governed by the polypeptide

encoded by the UGT1A1 gene. Therefore, in order to increase the predictability and/or efficiency of therapeutic measures applied in accordance with the present invention, the genetic constitution of a subject as regards the present or absence of the variant alleles referred to herein has to be determined and based on that knowledge an individual therapy can be developed which is therapeutically most effective and which avoids toxic or undesirable side effects caused by the substances according to the invention.

In a preferred embodiment of the use of the present invention said variant allele comprises a polynucleotide selected from the group consisting of:

- (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 37, 69 or 97;
- (b) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 558, 570 or 584;
- (c) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 890, 1117 or 1471 of the UGT1A1 gene (Accession No: GI: 8850235);
- (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having an A at a position corresponding to position 1117, a T at a position corresponding to position 890 or a G at a position corresponding to position 1471 of the UGT1A1 gene (Accession No: GI:8850235);
- (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution at a position corresponding to position 292, 368 or 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236); and
- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises amino acid substitution of Ala to Val at a position corresponding to position 292, Ala to Thr at a position corresponding

to position 368 or Tyr to Asp at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236).

More preferably, said fourth variant allele comprises a polynucleotide selected from the group consisting of:

- (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 97;
- (b) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 584;
- (c) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 1471 of the UGT1A1 gene (Accession No: GI: 8850235);
- (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having a G at a position corresponding to position 1471 of the UGT1A1 gene (Accession No: GI:8850235);
- (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236); and
- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises amino acid substitution of Ala to Thr at a position corresponding to position 368 or Tyr to Asp at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236).

The present invention also relates to a method of treating or preventing colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer comprising:



- (a) determining the presence or absence of a variant allele comprising a polynucleotide referred to herein; and
- (b) administering to a subject a therapeutically effective dosage of irinotecan.

The definitions used in accordance with the use of the present invention apply mutatis mutandis to the above method. Further, all embodiments described in accordance with the use of the present invention can be applied mutatis mutandis to the method of the present invention. Moreover, also encompassed by the method of the present invention are any further developments of said method which the person skilled in the art can make without undue burden based on its knowledge and the prior art, such as those documents referred to throughout this specification.

In a preferred embodiment of the use of the present invention a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered expression of the variant allele compared to the corresponding wild type allele.

As discussed above, the alleles referred to in accordance with the use of the present invention correspond to the UGT1A1 gene. It is well known in the art that genes comprise structural elements which encode an amino acid sequence as well as regulatory elements which are involved in the regulation of the expression of said genes. Structural elements are represented by exons which may either encode an amino acid sequence or which may code for RNA which is not encoding an amino acid sequence but is nevertheless involved in RNA function, e.g. by regulating the stability of the RNA or the nuclear export of the RNA.

Regulatory elements of a gene may comprise promoter elements or enhancer elements both of which could be involved in transcriptional control of gene expression. It is very well known in the art that a promoter is to be found upstream of the structural elements of a gene. Regulatory elements such as enhancer elements, however, can be found distributed over the entire locus of a gene. Said elements could reside, e.g., in introns, regions of genomic DNA which separate the exons of a gene. Promoter or enhancer elements correspond to polynucleotide fragments which are capable of attracting or binding polypeptides involved in the

regulation of the gene comprising said promoter or enhancer elements. For example, polypeptides involved in regulation of said gene comprise the so called transcription factors.

Said introns may comprise further regulatory elements which are required for proper gene expression. Introns are usually transcribed together with the exons of a gene resulting in a nascent RNA transcript which contains both, exon and intron sequences. The intron encoded RNA sequences are usually removed by a process known as RNA splicing. However, said process also requires regulatory sequences present on a RNA transcript said regulatory sequences may be encoded by the introns.

In addition, besides their function in transcriptional control and control of proper RNA processing and/or stability, regulatory elements of a gene could be also involved in the control of genetic stability of a gene locus. Said elements control, e.g., recombination events or serve to maintain a certain structure of the DNA or the arrangement of DNA in a chromosome.

Therefore, single nucleotide polymorphisms can occur in exons of an allele of a gene which encode an amino acid sequence as discussed supra as well as in regulatory regions which are involved in the above discussed process. The polymorphisms comprised by the polynucleotides referred to in accordance with the use of the present invention can influence the expression level of UGT1A1 protein via mechanisms involving enhanced or reduced transcription of UGT1A1 gene, stabilization of the gene's RNA transcripts and alteration of the processing of the primary RNA transcripts.

Methods for the determination of an altered expression of a variant allele when compared to its wild type counterpart are well known in the art and comprise inter alia those referred to herein above, e.g., PCR based techniques, RFLP-based techniques, DNA sequencing-based techniques, hybridization techniques, Single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), mismatch cleavage detection, heteroduplex analysis, techniques based on mass spectroscopy, HPLC-based techniques, primer extension-based techniques, and 5'-nuclease assay-based techniques. It might be necessary to obtain a sample comprising biological material, such as isolated cells

or tissue from the subject prior to perform said methods for determination of the expression levels of the wild type and the variant alleles, respectively. An altered expression in accordance with the use of the present invention means that the expression of the wild type allele differs significantly from the expression of the variant allele. A significant difference can be determined by standard statistical methods, such as Student's t-test,  $\chi^2$ -test or the U-test according to Mann and Whitney. Moreover, the person skilled in the art can adopt these and other statistical method known in the art individually without an undue burden.

In a more preferred embodiment of the use of the invention said altered expression is decreased or increased expression.

To determine whether the expression of an allele referred to in accordance to the present invention is increased or decreased in comparison to the corresponding wild type allele well known methods such as PCR based techniques, RFLP-based techniques, DNA sequencing-based techniques, hybridization techniques, Single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), mismatch cleavage detection, heteroduplex analysis, techniques based on mass spectroscopy, HPLC-based techniques, primer extension-based techniques, and 5'-nuclease assay-based techniques can be applied. As discussed above, it might be necessary to obtain a sample comprising cells or tissue from the subject in order to determine the expression level of the variant allele referred to in the use of the invention. A decrease or increase of the expression is characterized by a significant difference in the expression level of the variant versus the wild type allele in those assays. Also encompassed by decreased expression is the absence detectable expression of a variant allele.

In a furthermore preferred embodiment of the use of the present invention a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered activity of the polypeptide encoded by the variant allele compared to the polypeptide encoded by the corresponding wild type allele.

As discussed supra, the variant alleles comprising those polynucleotides specified herein which correspond to coding regions of the UGT1A1 gene effect the amino acid sequences of the polypeptides encoded by said variant alleles. The variant polypeptides, therefore, exhibit altered biological and/or immunological properties when compared to their corresponding wild type counterpart. Preferred variant polypeptides in accordance with the use of the invention are those, which exhibit an altered biological activity, i.e. altered enzymatic function resulting in reduced, enhanced or complete loss of catalytic activity or altered transport function resulting in reduced, enhanced or complete loss of transport activity or altered binding to receptors or other drug targets resulting in altered activation of signal transduction pathways or altered inhibition of transporter or enzyme function. It might be necessary to obtain a sample comprising biological material such as isolated cells or tissue from the subject prior to perform said methods for determination of the activities of the wild type and the variant polypeptides, respectively. Whether a variant polypeptide has an altered activity or level of expression compared to its wild type corresponding counterpart can be determined by standard techniques well known in the art. Such standard techniques may comprise, e.g., ELISA based assays, RIA based assays, HPLC-based assays, mass spectroscopy-based assays, western blot analysis or assays which are known in the art and described in [Ciotti, *et al.*, 1999, Biochem Biophys Res Commun 260:199-202, Lyer, *et al.*, 1999, Clin Pharmacol Ther 65:576-82, Iolason, *et al.*, 2000, J Med Genet 37:712-3, Raijmakers, *et al.*, 2000, J Hepatol 33:348-51, von Ahsen, *et al.*, 2000, Clin Chem 46:1939-45, Beutler, *et al.*, 1998, Proc Natl Acad Sci U S A 95:8170-4, Kadakol, *et al.*, 2000, Hum Mutat 16:297-306].

An altered activity in accordance with the use of the present invention means that the activity of the wild type polypeptide differs significantly from the variant polypeptide. A significant difference can be determined by standard statistical methods referred to herein above.

Most preferably, said altered activity is decreased or increased activity.

As discussed for the increase or decrease of expression, a decrease or increase of the activities is characterized by a significant difference in the activity of the variant versus the wild type polypeptide in the assays referred to herein. Also encompassed by decreased activity is the absence detectable activity of a variant allele.

Moreover, in a further preferred embodiment of the use of the present invention said subject is an animal.

As described supra, the subject in accordance with the use of the present invention encompasses animals. The term "animal" as used herein encompasses all animals, preferably animals belonging to the vertebrate family, more preferably mammals. Moreover, the animals can be genetically engineered by well known techniques comprising transgenesis and homologous recombination in order to incorporate one or more of the polynucleotides referred to supra into the genome of said animals. Said animals comprising the genetically engineered animals can be used to study the pharmacological effects of drugs or pro-drugs which are based on the substances or derivatives thereof referred to herein, preferably irinotecan.

In accordance with the foregoing, most preferably, said animal is a mouse or rat. Said animals are particularly well suited for assaying the pharmacological properties of the substances or derivatives referred to in accordance with the use of the present invention as described in detail in Giovanella, *et al.*, 1991, Cancer Res 51:3052-5, Kunimoto, *et al.*, 1987, Cancer Res 47:5944-7, Kaneda, *et al.*, 1990, Cancer Res 50:1715-20.

Preferably, said mouse is lacking functional UGT1A1. It is well known in the art how said mice lacking functional UGT1A1 can be obtained. For instance said mice might be generated by homologous recombination as described for cytochrome P450 in Pineau, *et al.*, 1998, Toxicol Lett 103:459-64, MRP1 in Rappa, *et al.*, 2000, Biochemistry 39:3304-10, and MDR1 in Schinkel, 1998, Int J Clin Pharmacol Ther 36:9-13, Schinkel, *et al.*, 2000, Pharmacogenetics 10:583-90.

Moreover, in another preferred embodiment of the use of the present invention said subject is a human.

In particular, the present invention is applicable to humans as is evident from the above. The use of the present invention is to be applied in order to treat side effects in patients which suffer from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer. The pharmacological effects of the above substances or derivatives thereof are well described in humans. However, the conventional therapies do not take into account the individual genetic makeup of the patient. Ethnic populations have different genetic backgrounds, which can also influence the function or regulation of a variant allele and thereby alter the pharmacological response of a patient to a substance or derivative used as a basis for a drug or pro-drug in accordance with the invention.

In light of the foregoing, most preferably, said human is African or Asian. The Asian population (16 %) who shows compared to Caucasians (39 %) a lower frequency of the UGT1A1 low expressor genotype (homozygously wildtype at positions corresponding to positions 174990 to 174993 of the UGT1A1 gene Acc. No. GI:11118740) and is therefore less likely to suffer from irinotecan toxicity. On the other hand, this allele is more common in Africans (43 %) who have additionally another low expressor allele (insertion of TA at positions corresponding to positions 174989/174990 of the UGT1A1 gene Acc. No. GI:11118740) the homozygous genotype of which occurs in 7 %. Africans are therefore more susceptible to irinotecan-related adverse events (population frequency data are from [Beutler, *et al.*, 1998, *Proc Natl Acad Sci U S A* 95:8170-4, Lampe, *et al.*, 1999, *Pharmacogenetics* 9:341-9, Hall, *et al.*, 1999, *Pharmacogenetics* 9:591-9]).

The present invention also relates to a method for selecting a suitable therapy for a subject suffering from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer, wherein said method comprises:

- (a) determining the presence or absence of a variant allele referred to above in the genome of a subject in a sample obtained from said subject; and

- (b) selecting a suitable therapy for said subject based on the results obtained in (a).

The definitions and explanations of the terms made above apply *mutatis mutandis* to the above method.

The term "suitable therapy" as used herein means that a substance according to the invention is selected and said substance being administered in a certain dosage to a subject, wherein said substance and said dosage are selected based on the knowledge of the presence or absence of a variant allele referred to in accordance with the use of the invention. Said substance and said dosage of the substance are selected in a way that on one hand they are most effective in treating colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer on the other hand they do not cause toxic or undesirable side effects.

As is evident from the above, a prerequisite for selecting a suitable therapy is the knowledge of the presence or absence of a variant allele referred to in accordance with the use of the invention. Therefore, the method of the present invention encompasses the determination of the presence or absence of said variant alleles in a sample which has been obtained from said subject. The sample which is obtained by the subject comprises biological material which is suitable for the determination of the presence or absence of said variant alleles, such as isolated cells or tissue. Methods for the determination of the presence or absence of the variant alleles of the method of the invention comprise those methods referred to herein above.

Thanks to the method of the present invention, it is possible to efficiently select a suitable therapy for a subject, preferably a human, suffering from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer. Thereby, mistreatment of patients based on wrong medications and the results thereof, such as development of resistance towards cancer therapy, and subsequent increased costs in health care, can be efficiently avoided. Furthermore, patients that are at high risk can be excluded from therapy prior to the

first dose and/or dosage can be adjusted according to the individual's genetic makeup prior to the onset of drug therapy. Also, inhibitors for the mentioned UGT1A1 gene can be applied in genetically defined patient subpopulations. Thus, adverse effects can be avoided and the optimal drug level can be reached faster without time-consuming and expensive drug monitoring-based dose finding. This can reduce costs of medical treatment and indirect costs of disease (e.g. shorter time and less frequent hospitalization of patients).

The following 29 items are also encompassed by the present invention. The definitions and explanations made supra apply mutatis mutandis to the terms used to characterize the claims.

1. A method of using irinotecan to treat a patient suffering from cancer which comprises:
  - (a) determining if the patient has one or more variant alleles of the UGT1A1 gene;
  - (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.
2. The method of item 1 wherein the cancer is colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, or pancreatic cancer.
3. The method of item 2 in which:
  - (a) the one or more variant alleles result in the patient expressing low amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is decreased to avoid toxicity; or



- (b) the one or more variant alleles result in the patient expressing high amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is increased to enhance efficacy.
- 4. The method of item 3 wherein the one or more variant alleles are in the promoter region of the UGT1A1 gene.
- 5. The method of item 3 wherein the one or more variant alleles are in the coding region of the UGT1A1 gene.
- 6. The method of item 3 wherein the one or more variant alleles are not in either the promoter region or the coding region of the UGT1A1 gene.
- 7. The method of item 3 wherein the one or more variant alleles are in both the promoter region and the coding region of the UGT1A1 gene.
- 8. The method of item 3 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:
  - (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 001, 002, 005, 006, 009, 010, 013, 014, 017, 018, 021, 022, 025, 026, 029, 030, 033, 034, 037, 038, 041, 042, 045, 046, 049, 050, 053, 054, 057, 058, 061, 062, 065, 066, 069, 070, 073, 074, 077, 078, 081, 082, 085, 086, 089, 090, 093, 094, 097, 098, 101, 102, 105, 106, 109, 110, 113, 114, 129, 130, 133 and/or 134;
  - (b) a polynucleotide encoding a polypeptide having the amino acid sequence of any one of SEQ ID NOs: 538, 540, 542, 544, 546, 548, 550,

552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596 and/or 598;

- (c) a polynucleotide capable of hybridizing to a Uridine Diphosphate Glycosyltransferase1 Member A1 (UGT1A1) gene, wherein said polynucleotide is having at a position corresponding to positions 59, 160, 226, 539, 544, 640, 701, 841, 855, 890, 938, 1006, 1007, 1020, 1084, 1085, 1114, 1117, 1139, 1158, 1175 to 1176, 1216, 1297, 1324, 1471, 1478, 372 to 373, 523 to 525, and/or 892 to 905 of the UGT1A1 gene (Accession No. GI:8850235), a substitution or deletion of at least one nucleotide or at a position corresponding to positions 470/471, and/or 1222/1223 of the UGT1A1 gene (Accession No. GI:8850235) a insertion of at least one nucleotide;
- (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having at a position corresponding to position 226, 539, 701, 855, 938, 1020, and/or 1117 of the UGT1A1 gene (Accession No: GI:8850235) an A, at a position corresponding to position 160, 640, 890, 1006, 1084, 1139, 1176, 1324, and/or 1478 of the UGT1A1 gene (Accession No: GI: 8850235) a T, at a position corresponding to position 544, 841, and/or 1216 of the UGT1A1 gene (Accession No: GI: 8850235) a C, at a position corresponding to position 59, 1007, 1085, 1114, 1158, 1175, 1297, and/or 1471 of the UGT1A1 gene (Accession No: GI:181303) a G, and/or at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TTC, at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TACATTAATGCTTC, at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a T, and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a G;

- (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution of Leu to Arg at a position corresponding to position 15 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 71 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Leu to Gln at a position corresponding to position 175 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Cys to Arg at a position corresponding to position 177 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Trp at a position corresponding to position 209 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Gln at a position corresponding to position 229 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 276 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Val at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Trp at a position corresponding to position 293 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Glu at a position corresponding to position 308 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding to position 331 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding to position 357 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Gly at a position corresponding to position 367 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Thr at a position corresponding to position 368 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Arg at a position corresponding to position 387 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a position corresponding to position 375 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Arg at a position corresponding to position 381 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Pro at a position corresponding to position 401 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Lys to Glu at a position corresponding to position 428 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Asp at a position

corresponding to position 486 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a position corresponding to position 488 of the UGT1A1 polypeptide (Accession No: G8850236);

- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide is having at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, whereby in said polypeptide one or more aminoacids following amino acid Asp at a position corresponding to position 119 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a T, whereby in said polypeptide one or more aminoacids following amino acid Pro at a position corresponding to position 152 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TTC, whereby in said polypeptide one or more aminoacids following amino acid Thr at a position corresponding to position 168 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TACATTAATGCTTC, whereby in said polypeptide one or more aminoacids following amino acid Ala at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a G, whereby in said polypeptide one or more aminoacids following amino acid Lys at a position corresponding to position 402 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted; and
- (g) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide comprises an amino acid substitution of Gln to a stop codon at a position corresponding to position 49 of the UGT1A1

gene (Accession No: G8850236) and/or an amino acid substitution of Cys to a stop codon at a position corresponding to position 280 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 331 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Trp to a stop codon at a position corresponding to position 335 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 357 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Lys to a stop codon at a position corresponding to position 437 of the UGT1A1 gene (Accession No: G8850236).

9. The method of item 8 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:
- (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 37, 69 or 97;
  - (b) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 558, 570 or 584;
  - (c) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 890, 1117 or 1471 of the UGT1A1 gene (Accession No: GI: 8850235);
  - (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having an A at a position corresponding to position 1117, a T at a position corresponding to position 890 or a G at a position corresponding to position 1471 of the UGT1A1 gene (Accession No: GI:8850235);
  - (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution at a

position corresponding to position 292, 368 or 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236); and

- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises amino acid substitution of Ala to Val at a position corresponding to position 292, Ala to Thr at a position corresponding to position 368 or Tyr to Asp at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236).

10. The method of item 8 in which the one or more variant alleles results in the patient expressing low amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is decreased.
11. The method of item 8 in which the one or more variant alleles results in the patient expressing high amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is increased.
12. The method of item 9 in which the one or more variant alleles results in the patient expressing low amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is decreased.
13. The method of item 9 in which the one or more variant alleles results in the patient expressing high amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is increased.
14. A method for determining whether a patient is at risk for a toxic reaction to treatment with irinotecan which comprises determining if the patient has one or more variant alleles of the UGT1A1 gene.

15. The method of item 14 which further comprises administering to the patient reduced amounts of irinotecan if the patient has one or more variant alleles that result in decreased expression of the UGT1A1 gene.
16. A method for determining the optimum treatment regimen for administering irinotecan to a patient suffering from cancer which comprises:
  - (a) determining if the patient has one or more variant alleles of the UGT1A1 gene;
  - (b) in a patient having one or more of such alleles increasing or decreasing the amount of irinotecan in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.
17. A method of treating cancer in a patient having one or more variant alleles of the UGT1A1 gene such that expression levels of the UGT1A1 gene product are lower than in the general population and so indicates high sensitivity to irinotecan which comprises administering to the patient a decreased amount of irinotecan.
18. A method of treating cancer in a patient having one or more variant alleles of the UGT1A1 gene such that expression levels of the UGT1A1 gene product are higher than in the general population and so indicates resistance or predisposition to resistance to irinotecan which comprises administering to the patient an increased amount of irinotecan.
19. The method of item 18 in which patients that have a variant allele that indicates resistance or predisposition to resistance are treated with an UGT1A1 inhibitor.
20. The method of item 19 wherein the UGT1A1 inhibitor is selected from the group consisting of:  $\beta$ -estradiol, 4-hydroxyestrone, 2-hydroxyestrone, 7,8-Benzoflavone, Quercetin, Naringenin, Chrysin, Bilirubin, and Octylgallate.

21. The method of item 17 which further comprises monitoring the patient during treatment by assaying for changes in expression levels of the UGT1A1 gene product in the cancerous cells whereby an increase in the expression level of the UGT1A1 gene product is compensated for by an increase in the amount of irinotecan administered to the patient.
22. A method of treating cancer in a patient which comprises internally administering to the patient an effective amount of irinotecan, wherein the treatment regimen is modified based upon the genotype of the patient's UGT1A1 gene.
23. A method of treating a population of patients suffering from cancer which comprises:
- (a) determining, on a patient by patient basis, if the patient has one or more variant alleles of the UGT1A1 gene;
  - (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.
24. A method of using irinotecan to treat a patient having Gilbert Syndrome who is suffering from cancer which comprises:
- (a) determining if the patient has one or more variant alleles of the UGT1A1 gene which results in low production or glucuronidation activity of the corresponding protein;
  - (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which amount is decreased in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.



25. A method of treating cancer in a patient having Gilbert Syndrome which comprises internally administering to the patient an effective amount of irinotecan, wherein the treatment regimen is modified based upon the genotype of the patient's UGT1A1 gene.
26. A method for predicting sensitivity to irinotecan in a patient suffering from cancer which comprises determining if the patient has one or more variant alleles of the UGT1A1 gene, which alleles indicate that the cancerous cells express low or high amounts of the UGT1A1 protein, whereby low expression indicates high sensitivity to irinotecan and high expression indicates resistance or predisposition to resistance to irinotecan.
27. The method of item 26 in which patients that have a genotype that indicates resistance or predisposition to resistance are treated with a UGT1A1 inhibitor.
28. The method of item 27 wherein the UGT1A1 inhibitor is selected from the group consisting of:  $\beta$ -estradiol, 4-hydroxyestrone, 2-hydroxyestrone, 7,8-Benzoflavone, Quercetin, Naringenin, Chrysin, Bilirubin, and Octylgallate.
29. The method of item 26 wherein the patients that have a genotype that indicates resistance or predisposition to resistance are monitored during treatment by assaying for expression levels of the UGT1A1 gene product in the cancerous cells.

The decreased expression as referred to herein above includes in addition to a significantly decreased amount of transcripts encoding a functional gene product also a normal or even elevated amount of transcripts encoding a gene product which has no activity or a significantly decreased activity.

By "in comparison to the amount that is administered without regard to the patient's alleles in the MDR1 gene" a standard dose is meant which is routinely administered to patients in need thereof without regarding the genotype. Such a general population of patients is considered as having the normal genotype, i.e. wildtype genotype.

Further, the present invention encompasses a method for improving and/or modifying a therapy comprising determining the expression level of UGT1A1, hereinafter referred to as expression profile or the protein level of the UGT1A1 protein, hereinafter referred to as the protein profile, or the activity level of the said proteins, hereinafter referred to as the activity profile.

The term "expression level" as referred to in the context of the present invention means the detectable amount of transcripts of the UGT1A1 gene relative to the amount of transcripts for a housekeeping gene, such as PLA2. The amount of transcripts can be determined by standard molecular biology techniques including Northern analysis, RNase protection assays, PCR based techniques encompassing Taq-Man analysis. Preferably, the determination can be carried out as described in the accompanied Examples 4 and 5. The term "expression profile" means that the expression level of a panel of the aforementioned genes is determined and the expression levels are compared to a reference standard. As a reference standard, preferably transcripts are obtained from cells or tissues of a subject having the aforementioned wildtype alleles of the respective genes in their genomes.

The term "protein level" refers to the detectable amount of UGT1A1 relative to the amount of a protein encoded by a housekeeping gene, such as PLA2. The amount of proteins can be determined by standard biochemical techniques, such as Western analysis, ELISA, RIA or other antibody based techniques known in the art. The term "protein profile" means that the protein level of a panel of the aforementioned proteins is determined and the protein levels are compared to a reference standard. As a reference standard, preferably proteins are obtained from cells or tissues of a subject having the aforementioned wildtype alleles of the respective genes in their genomes.

The term "activity level" means the detectable biological activity of UGT1A1 relative to the activity or amount of a encoded by the allelic variants of these genes as disclosed in the present invention relative to the activity compared to a suitable

reference standard (e.g. of the protein encoded by the corresponding wild-type allele of the gene). Biological assays for the aforementioned proteins are well known in the art and described in Hitzl *et al.*, 2001, *Pharmacogenetics* 11:293-8, Cuff *et al.*, *Toxicol Lett.*, 2001, 120:43-9, Stevens *et al.*, *Drug Metab Dispos.*, 2001, 29:289-95, Barbier *et al.*, *Mol Pharmacol.*, 2001, 59:636-45, Hanioka *et al.*, *Xenobiotica*, 2001, 31:687-99, Hallo *et al.*, *Anticancer Res.* 1998, 18:2981-7. As a reference standard, preferably proteins are obtained from cells or tissues of a subject having the aforementioned wildtype alleles of the respective genes in their genomes.

The aforementioned methods, preferably, comprise the steps (i) obtaining a tumor sample from a patient during specific stages of a tumor therapy; and (ii) determining the expression profile, protein profile or activity profile for UGT1A1. Based on the expression profiles a clinician can efficiently adapt the therapy. This comprises inter alia dosage adjustment and/or including administration of an UGT1A1 inhibitor. Preferably, said inhibitor is selected from the following group of inhibitors:  $\beta$ -estradiol, 4-hydroxyestrone, 2-hydroxyestrone, 7,8-Benzoflavone, Quercetin, Naringenin, Chrysin, Bilirubin, Octylgallate (Broudy M (2001), BD Gentest, Woburn MA, USA).

The term inhibitor as used herein encompasses competitive and non-competitive inhibitors.

Finally, the present invention encompasses a method for determining whether a patient has developed a resistance against the drugs referred to in the context of the present invention. Said method comprising the steps of (i) obtaining a tumor sample from a patient during specific stages of a tumor therapy; and (ii) determining the expression level UGT1A1. The expression of the respective genes can be determined as described in Examples 4 and 5 or as described above. Based on the evaluation of said expression profile, a clinician can more efficiently adapt the therapy. This comprises inter alia dosage adjustment and/or including administration of an UGT1A1 inhibitor as defined supra.

Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference.

The nucleic acid and amino acid sequences referred to in this application by sequence identification numbers (SEQ ID NOs.) are listed in the following Tables 1, 2, 3 and 4. For positions of polymorphic nucleotides, the following substitute letters are used in the nucleic acid sequences: R, G or A; Y, T or C; M, A or C; K, G or T; S, G or C; W, A or T.

Amino acid sequences are shown in the one letter code. The letter X at polymorphic amino acid positions represents the modified amino acid or its corresponding wild type amino acid (see accession numbers).

Moreover, all nucleic acid and amino acid sequences referred to herein by making reference to GenBank accession numbers are shown in Figures 4 to 29 below.

Table 1: The nucleic acid and amino acid sequences referred to in this application

Gene	Variati on	SNP	Acc.no.	SEQ		Sequence		SEQ		Sequence		SEQ		Sequence wt>mut	
				ID	No	forward	ID	No	reverse	ID	No	forward	ID	No	reverse
UGT1A1	T>G	59	GI:8850235	001	GTCCTGGGCCG	002	ACACAGCAGCC	003	GTCCTGGGCCCK	004	ACACAGCAGCM				
					GCTGCTGTGT		GGCCCAGGAC		GCTGCTGTGT		GGCCCAGGAC				
UGT1A1	C>T	160	GI:8850235	005	GGCCATCCAGI	006	TGCTGCAGCTA	007	GGCCATCCAGY	008	TGCTGCAGCTR				
					AGCTGCAGCA		CTGGATGGCC		AGCTGCAGCA		AGCTGCAGCA				
UGT1A1	G>A	226	GI:8850235	009	CATCAGAGACA	010	TAAATGCTCIG	011	CATCAGAGACR	012	TAAATGCTCYG				
					GAGCATTTTA		TCTCTGATG		GAGCATTTTA		TCTCTGATG				
UGT1A1	T>A	539	GI:8850235	013	TTGCATGCACA	014	GCTGCATGGCI	015	TTGCATGCACW	016	GCTGCATGGCK				
					GCCATGCAGC		GTGCATGCAA		GCCATGCAGC		GTGCATGCAA				
UGT1A1	T>C	544	GI:8850235	017	TGCACTGCCAC	018	TCCAGGCTGCC	019	TGCACTGCCAY	020	TCCAGGCTGCR				
					GCAGCCTGGA		GTGCATGCAA		GCAGCCTGGA		GTGCATGCAA				

UGT1A1 C>T 640 GI:8850235 021 CTTCTGCAGT 022 TTCTTACCCAC 023 CTTCTGCAGY 024 TTCTTACCCRC  
 GGTGAAGAA TGCAGGAAG GGTGAAGAA TGCAGGAAG  
 UGT1A1 C>A 701 GI:8850235 025 GTTTATTCCAG 026 GGTGCATACI 027 GTTTATCCCM 028 GGTGCATACK  
 TATGCAACC GGAATAAAC GTATGCAACC GGAATAAAC  
 UGT1A1 G>C 841 GI:8850235 029 GGTTTTGTTCG 030 TTGATTCCACG 031 GGTTTTGTTS 032 TTGATTCCACSA  
 TGGAAATCAA AACAAAAACC TGGAAATCAA AAAAAACC  
 UGT1A1 C>A 855 GI:8850235 033 GAATCAACTGA 034 TTTGGTGAAGT 035 GAATCAACTGM 036 TTTGGTGAAGK  
 CTTCAACCAA CAGTTGATTC CTTCAACCAA CAGTTGATTC  
 UGT1A1 C>T 890 GI:8850235 037 GAATTGGAAGTC 038 ATTAATGTAGAC 039 GAATTGGAAGY 040 ATTAATGTAGRC  
 TACATTAAT TTCAAATTC CTACATTAAT TTCAAATTC  
 UGT1A1 G>A 938 GI:8850235 041 TTCTCTTTGGA 042 GACCAATTGATTC 043 TTCTCTTTGGR 044 GACCAATTGATY  
 TCAATGGTC CAAAGAGAA TCAATGGTC CCAAGAGAA  
 UGT1A1 C>T 1006 GI:8850235 045 CAAAATCCCTTA 046 AGGACTGTCTA 047 CAAAATCCCTYA 048 AGGACTGTCTR  
 GACAGTCCT AGGGATTTTG GACAGTCCT AGGGATTTTG  
 UGT1A1 A>G 1007 GI:8850235 049 AAAATCCCTGG 050 CAGGACTGTCC 051 AAAATCCCTCR 052 CAGGACTGTCTY  
 GACAGTCCTG GAGGGATTTT GACAGTCCTG GAGGGATTTT  
 UGT1A1 G>A 1020 GI:8850235 053 CAGTCCTGTGA 054 CAGTGACCGT 055 CAGTCCTGTGR 056 CAGTGATACCGY

UGT1A1 C>T 1084	GI:8850235	057 GTGGCTACCC <u>I</u>	058 AGATCGTTTT <u>A</u> G	059 GTGGCTACCC <u>Y</u>	060 AGATCGTTTT <u>R</u>
		AAACGATCT	GGTAGCCAC	AAACGATCT	GGGTAGCCAC
UGT1A1 A>G 1085	GI:8850235	061 TGGCTACCC <u>G</u>	062 CAGATCGTTT <u>C</u>	063 TGGCTACCC <u>R</u>	064 CAGATCGTTT <u>Y</u>
		AAACGATCTG	GGGTAGCCA	AAACGATCTG	GGGTAGCCA
UGT1A1 C>G 1114	GI:8850235	065 CCCGATGACC <u>G</u>	066 ATAAAGGCAC <u>C</u>	067 CCCGATGACC <u>S</u>	068 ATAAAGGCAC <u>S</u>
		GTGCCTTTAT	GGTCATCGGG	GTGCCTTTAT	GGTCATCGGG

UGT1A1 G>A 1117 GI:8850235 069 GATGACCCGTA 070 GTGATAAAGGI 071 GATGACCCGTR 072 GTGATAAAGGY  
 CCTTTATCAC ACGGGTCATC CCTTTATCAC ACGGGTCATC  
 UGT1A1 C>T 1139 GI:8850235 073 CATGCTGGTTI 074 AACACCATGGA 075 CATGCTGGTTY 076 AACACCATGGR  
 CCATGGTGTT AACAGCATG CCATGGTGTT AACAGCATG  
 UGT1A1 C>G 1158 GI:8850235 077 TTTATGAAAGGA 078 CATTGCATATCC 079 TTTATGAAAGSA 080 CATTGCATATSC  
 TATGCAATG TTTCATATAA TATGCAATG TTTCATATAA  
 UGT1A1 CC> 1175 to GI:8850235 081 AATGGCGTTTCG 082 TCATCACCATAC 083 AATGGCGTTCTY 084 TCATCACCATSR  
 GT 1176 IATGGTGATGA GAACGCCATT ATGGTGATGA GAACGCCATT  
 UGT1A1 G>C 1216 GI:8850235 085 GATGGACAATC 086 ATGCGCTTTGG 087 GATGGACAATS 088 ATGCGCTTTGS  
 CAAAGCGCAT ATTGTCCATC CAAAGCGCAT ATTGTCCATC  
 UGT1A1 A>G 1297 GI:8850235 089 AAATGCTCTAGA 090 ATGACTGCTTCT 091 AAATGCTCTARA 092 ATGACTGCTTYT  
 AGCAGTCTAT AGAGCATTT AGCAGTCTAT AGAGCATTT  
 UGT1A1 A>T 1324 GI:8850235 093 CAAAAGTTACTA 094 ATGTTCTCTAG 095 CAAAAGTTACW 096 ATGTTCTCTTW  
 GGAGAACAT TAACITTTTG AGGAGAACAT GTAACITTTTG  
 UGT1A1 T>G 1471 GI:8850235 097 CTGGTACCAGG 098 AAGGAATGGTC 099 CTGGTACCAGK 100 AAGGAATGGTM  
 ACCATTCTCT CTGGTACCAG ACCATTCTCT CTGGTACCAG



UGT1A1 C>T 1478 GI:8850235 101 CAGTACCATTTC 102 CACGTCCAAGA 103 CAGTACCATTYC 104 CACGTCCAAGR  
 TTGGACGTG AATGGTACTG TTGGACGTG AATGGTACTG  
 UGT1A1 deCT 372 toGI:8850235 105 TAAAAAAGGAC 106 AGCATAGCAGT 107 TAAAAAAGGANC 108 AGCATAGCAGn  
 TGCTATGCT CCTTTTTTA TGCTATGCT TCCTTTTTTA  
 UGT1A1 del 523 toGI:8850235 109 GCCCACTGTAT 110 CATGCAAGAAT 111 GCCCACTGTAn 112 CATGCAAGAAnt  
 TTC 525 TCTTGCAATG ACAGTGGGC TTCTTGCAATG ACAGTGGGC  
 UGT1A1 del 892 toGI:8850235 113 ATTTGAAGCCT 114 ATGTTCTCCAG 115 ATTTGAAGCCnT 116 ATGTTCTCCAnG  
 905 GGAGAACAT GCTTCAAAT GGAGAACAT GCTTCAAAT  
 TACA  
 TTA  
 ATGC  
 TTC  
 UGT1A1 insT 470/ GI:8850235 129 CTGACGGACCC 130 AAGGAAGGAAA 131 CTGACGGACCC 132 AAGGAAGGAAA  
 471 ITTCTTCTT TGGTCCGTCA nTTCTTCTT nGGGTCCGTCA  
 G  
 UGT1A1 insG 1222/1 GI:8850235 133 CAATGCAAAGC 134 AGTCTCCATGC 135 CAATGCAAAGC 136 AGTCTCCATGC  
 223 GGCATGGAGAC CGCTTTGCATT nGCATGGAGAC nGCTTTGCATTG  
 T G T



MRP1	C>T 18067 U91318	TCCCTGACCC	GACTTCCCAG	TCCCTGACCC	GACTTCCCAG
		193 CCACGGCAGCT	194 CCAGGTCCACA	195 CCACGGCAGCY	196 CCAGGTCCACR
		GTGGACCTGG	GCTGCCGTGG	GTGGACCTGG	GCTGCCGTGG
MRP1	G>A 79 AF022830	197 CCAGGCAGCCA	198 CAACCTTCACI	199 CCAGGCAGCCR	200 CAACCTTCACY
		GTGAAGGTTG	GGCTGCCTGG	GTGAAGGTTG	GGCTGCCTGG
MRP1	T>C 88 AF022830	201 CGGTGAAGTC	202 AGGAGTACACG	203 CGGTGAAGGTY	204 AGGAGTACACR
		GTGTACTCCT	ACCTTCACCG	GTGTACTCCT	ACCTTCACCG
MRP1	T>G 249 AF022830	205 CTCATGAGCTG	206 CTTGAAGAAGC	207 CTCATGAGCTK	208 CTTGAAGAAGM
		CTTCTTCAAG	AGCTCATGAG	CTTCTTCAAG	AGCTCATGAG
MRP1	T>C 95 AF022831	209 AGTTCGTGAAC	210 CCTTCGTGTGG	211 AGTTCGTGAAY	212 CCTTCGTGTGR
		GACACGAAGG	TTCACGAACT	GACACGAAGG	TTCACGAACT
MRP1	C>T 57853 GI:7209451	213 GGCAGTGGGCI	214 CCACTCCCTCA	215 GGCAGTGGGCY	216 CCACTCCCTCR
		GAGGGAGTGG	GCCCACTGCC	GAGGGAGTGG	GCCCACTGCC
MRP1	C>G 53282 GI:7209451	217 GCCAGTTGGAG	218 CCCCAAGTGAC	219 GCCAGTTGGAS	220 CCCCAAGTGAS
		TCACTTGGGG	TCCAACTGGC	TCACTTGGGG	TCCAACTGGC
MRP1	A>G 137710 AC026452	221 ACTCTCACTCG	222 TGCTGTGCCCC	223 ACTCTCACTCR	224 TGCTGTGCCCCY
		GGGCACAGCA	GAGTGAGAGT	GGGCACAGCA	GAGTGAGAGT

MRP1	G>C 27159	AC003026	225 TCGTTGATCA <u>CA</u>	226 ACAGACAGAT <u>G</u>	227 TCGTTGATCA <u>SA</u>	228 ACAGACAGAT <u>S</u>
			TCTGTCTGT	TGATCAACGA	TCTGTCTGT	TGATCAACGA
MRP1	G>A 34218	AC003026	229 GTGCACTCACA	230 CACCCGGCCAI	231 GTGCACTCAC <u>R</u>	232 CACCCGGCCAY
			TGGCCGGGTG	GTGAGTGCAC	TGGCCGGGTG	GTGAGTGCAC
MRP1	G>C 34215	AC003026	233 CATGTGCACT <u>C</u>	234 CCGGCCACGT <u>G</u>	235 CATGTGCACT <u>S</u>	236 CCGGCCACGT <u>S</u>
			ACGTGGCCGG	AGTGCACA <u>TG</u>	ACGTGGCCGG	AGTGCACA <u>TG</u>
MRP1	G>A 39508	GI:7209451	237 GTTTCGTTGTA	238 TCCACCCCCC <u>I</u>	239 GTTTCGTTGTR	240 TCCACCCCCY
			GGGGGTGGGA	ACAACGAAAC	GGGGGTGGGA	ACAACGAAAC
MRP1	T>C 55472	AC003026	241 TGTCTAATTACA	242 ATCCATTCTGT	243 TGTCTAATTAY <u>A</u>	244 ATCCATTCTCT <u>R</u>
			GAAATGGAT	AATTAGACA	GAAATGGAT	AATTAGACA
MRP1	G>A 150727	AC025277	245 CCATGTCAGCA	246 ACCTGTGTCA <u>I</u>	247 CCATGTGAGC <u>R</u>	248 ACCTGTGTCA <u>Y</u>
			TGACACAGGT	GCTGACATGG	TGACACAGGT	GCTGACATGG
MRP1	del/T 17970	U91318	249 CTGGTTTTT <u>ICT</u>	250 TGACCGGAAG <u>A</u>	251 CTGGTTTTT <u>TTTC</u>	252 TGACCGGAAG <u>n</u>
			TCCGGTCA	AAAAACCA <u>G</u>	TTCCGGTCA	AAAAAAACCA <u>G</u>
MRP1	C>T 17900	U91318	253 TGTCTCCTT <u>TTIG</u>	254 TGGGAGAGCA	255 TGTCTCCTT <u>TTYG</u>	256 TGGGAGAGC <u>R</u>
			CTTCTCCCA	AAAGGAGACA	CTTCTCCCA	AAAGGAGACA
MRP1	G>A 18195	U91318	257 CACTGGCACAA	258 CTAGAGGCCA <u>I</u>	259 CACTGGCACAR	260 CTAGAGGCCA <u>Y</u>

MRP1	G>A 33551	AC025277	261	TGTGACCACAA	262	ACACACTCATTT	263	TGTGACCACAR	264	ACACACTCATYT
				ATGAGTGTGT		GTGGTCACA		ATGAGTGTGT		GTGGTCACA
MRP1	C>T 174	AF022828	265	CCAGGCCCCCI	266	CCTGAGGTCTA	267	CCAGGCCCCCY	268	CCTGAGGTCTR
				AGACCTCAGG		GGGGGCCCTGG		AGACCTCAGG		GGGGGCCCTGG
MRP1	C>A 248	AF022829	269	CCTTTCCACTAC	270	GAGGCCACAGI	271	CCTTTCCACTM	272	GAGGCCACAGK
				TGTGGCCTC		AGTGGAAGG		CTGTGGCCTC		AGTGGAAGG
MRP1	C>G 258	AF022829	273	CCTGTGGCCTG	274	ATCCTGGATTG	275	CCTGTGGCCTS	276	ATCCTGGATTSA
				AATCCAGGAT		AGGCCACAGG		AATCCAGGAT		GGCCACAGG
MRP1	A>G 259	AF022831	277	AAGGTAGGGGG	278	TGGCACAGCGC	279	AAGGTAGGGGR	280	TGGCACAGCGY
				CGCTGTGCCA		CCCTACCTT		CGCTGTGCCA		CCCTACCTT
MRP1	T>C 124667	AC026452	281	GCGTGCCCAAGC	282	AAACCCCAAGG	283	GCGTGCCCAAGY	284	AAACCCCAAGR
				CCTGGGGTTT		CTGGGCACGC		CCTGGGGTTT		CTGGGCACGC
MRP1	G>A 1884	U07050	285	AGCCTTGAGA	286	CACCCAGATI	287	AGCCTTGAGR	288	CACCCAGATY
				ATCTGGGGTG		CTCCAAGGCT		ATCTGGGGTG		CTCCAAGGCT
MRP1	G>C 38646	AC026452	289	CCTTAAACAGC	290	CTTTTCAAATGC	291	CCTTAAACAGSA	292	CTTTTCAAATSC
				ATTTGAAAAG		TGTTTAAGG		TTTGAAAAG		TGTTTAAGG

MRP1	C>A 1625	U07050	293 GGAATCACTA 294 CAGAGAGGTTI 295 GGAATCACTM 296 CAGAGAGGTTK AACCTCTCTG AGTGATTCCC AACCTCTCTG	296 CAGAGAGGTTK AGTGATTCCC
MRP1	C>T 1163	U07050	297 TGTGATCGGCI 298 AGCCGAGGCGA 299 TGTGATCGGCY 300 AGCCGAGGCGR CGCCTCGGCT GCCGATCACA CGCCTCGGCT	300 AGCCGAGGCGR GCCGATCACA
MRP1	A>G 381	U07050	301 TGGGGACCCG 302 TTTATTGGCC 303 TGGGGACCC 304 TTTATTGGCCY GGCCAATAAA GGGTCCCCCA A RGGCCAATAA	304 TTTATTGGCCY GGGTCCCCCA
MRP1	G>A 233	U07050	305 AAGAGTAGCAA 306 CAAGATAAAIT 307 AAGAGTAGCA 308 CAAGATAAAAYT TTTTATCTTG GCTACTCTT RTTTTATCTTG	308 CAAGATAAAAYT GCTACTCTT
MRP1	C>A 189	U07050	309 AAAAAATCCAA 310 TTTTGGATTIG 311 AAAAAATCCM 312 TTTTGGATTKG ATCCAAAAA GATTTTTT AATCCAAAAA	312 TTTTGGATTKG GATTTTTT
MRP1	C>T 440	U07050	313 CTCCTTCCCTIG 314 AGGACCTAGCA 315 CTCCTTCCCTY 316 AGGACCTAGCR CTAGGTCCT AGGGAAGGAG GCTAGGTCCT	316 AGGACCTAGCR AGGGAAGGAG
MRP1	del/AT 34206 to 34207	AC003026	317 AGTCTCACACG 318 GTGAGTGCACG 319 AGTCTCACACn 320 GTGAGTGCACn TGCACCTCAC TGTGAGACT GTGCACTCAC	320 GTGAGTGCACn GTGTGAGACT
MRP1	delGG1720 to U07050		321 ACTCCAGGCAG 322 GAACGGAGGCTI 323 ACTCCAGGCA 324 GAACGGAGGCCn	324 GAACGGAGGCCn

TA 1723		GCTCCGTTT	GCCTGGAGT	GGCTCCGTT C	TGCCTGGAGT
MRP1	inst 926/92 U07050 7	325 TTAATTTTTTTT ATTATTATT	326 AAATAATAATAA AAAAAAATTAA	327 TTAATTTTTTTT ATTATTATT	328 AAATAATAATAA AAAAAAATTAA
MRP1	Inst 437/43 U07050 CTTC 8 C	329 TTCTCTCTTCTT CCTTCCCTCGC TAGGT	330 ACCTAGCGAGG GAAGGAGGAAG GAGGAA	331 TTCTCTCTTCC CTCTCGCTAGG T	332 ACCTAGCGAGA GGAAGGAGGAA
MRP1	instTG 55156/ AC003026 GGG C 55157	333 GGGGCTGGGG CTGGGGCTGGG TGCGTG	334 CACGCACCCCGA CCCCGACCCAG CCCC	335 GGGGCTGGGG CTGGGTGCG TG	336 CACGCACCCCG ACCCAGCCCC
MDR1	T>C 140837 AC002457	337 GCTCATTTCGAG CAGCGGCTCT	338 AGAGCCGCTGC TCGAATGAG	339 CTCATTTCGAGY AGCGGCTCTT	340 AGAGCCGCTRC TCGAATGAG
MDR1	G>A 84701 AC005068	341 AAAATTGCTATC ACTATCT	342 AGATAGTGATA GCAATTTT	343 AAAATTGCTRT CACTATCT	344 AGATAGTGAYA GCAATTTT
MDR1	G>A 101 M29432	345 TTCACTTCAATT ACCCATC	346 ATGGGTAAITG AAGTGAA	347 TCACTTCARIT ACCCATC	348 GATGGGTAAYT GAAGTGAA
MDR1	C>T 308 M29432	349 CTTGAAGGGTC TGAACCTGA	350 TCAGGTTTCAGA CCCTTCAAGA	351 TCTTGAAGGG YCTGAACCTG	352 TCAGGTTTCAGR CCCTTCAAGA

MDR1	C>T 83946	AC005068	353 TCAGCAGT <u>I</u> AC ATTGCA	354 TGCAATGTAACT GCTGA	355 CAGCAGT <u>Y</u> AC ATTGCAC	356 TGCAATGT <u>R</u> AC TGCTGA
MDR1	G>A 83973	AC005068	357 GACCCATGCAA GCTAGACC	358 GGTCTAGCT <u>I</u> G CATGGGTC	359 GACCCATG <u>C</u> R AGCTAGACC	360 GGTCTAGCT <u>Y</u> G CATGGGTC
MDR1	A>G 84032	AC005068	361 GAGCACAACGG TCCAGCTG	362 CAGCTGGAC <u>C</u> G TTGTGCTC	363 GAGCACAAC <u>R</u> GTCCAGCTG	364 CAGCTGGAC <u>Y</u> G TTGTGCTC
MDR1	G>A 84074	AC005068	365 TGGGCAGACAG TGGCCCTG	366 CAGGGCCAC <u>I</u> G TCTGCCCA	367 TGGGCAGAC <u>R</u> GTGGCCCTG	368 CAGGGCCAC <u>Y</u> G TCTGCCCA
MDR1	G>A 84119	AC005068	369 CTCGTCCTGAT AGATCTTG	370 CAAGATCTA <u>T</u> CA GGACGAG	371 CTCGTCCT <u>G</u> R TAGATCTTG	372 CAAGATCTA <u>Y</u> CA GGACGAG
MDR1	A>G 77811	AC005068	373 GGCTTGAAGGT GTAAGAAT	374 ATTCTTACA <u>C</u> CT TCAAGCC	375 GGCTTGAAG <u>R</u> TGTAAGAAT	376 ATTCTTACA <u>Y</u> CT TCAAGCC
MDR1	T>A 78170	AC005068	377 TATTCCTTTACA AATTTTIG	378 CAAAAATT <u>I</u> GTA AAGGAATA	379 TATTCCTTTAC WAAATTTTIG	380 AAAAAAATT <u>W</u> G TAAAGGAAT
MDR1	A>G 73252	AC005068	381 ACTTTGTCTGAT CTCCTGC	382 GCAGGAGAT <u>C</u> A GACAAAGT	383 ACTTTGTCT <u>R</u> A TCTCCTGC	384 GCAGGAGAT <u>Y</u> A GACAAAGT
MDR1	G>A 141529	AC002457	385 CTTCAGGTCGG	386 CAAGATCCAT <u>T</u> C	387 CTTCAGGTCG	388 CAAGATCCAT <u>Y</u>



		AATGGATCTTG	CGACCTGA	GRATGGATCTT	CCGACCTGAAG
		G			
MDR1	A>G 141590 AC002457	389 AA <u>ACTGAACGA</u>	390 TACCTTTTATCG	391 AA <u>ACTGAACRA</u>	392 TACCTTTTATYG
		TAAAGGTA	TTCAGTTTAA	TAAAGGTA	TTCAGTTTAA
MDR1	C>T 70200 AC005068	393 TTCTCCTTAIGG	394 CTAACACCCAT	395 TTCTCCTTAYG	396 CTAACACCCRT
		GTGTTAG	AAGGAGAA	GGTGTTAG	AAGGAGAA
MDR1	C>A 70204 AC005068	397 AATTTTCTCATT	398 CACCCGTAATG	399 AATTTTCTCMT	400 CACCCGTAAKG
		ACGGGTG	AGAAAATT	TACGGGTG	AGAAAATT
MDR1	C>T 70237 AC005068	401 TTAATTGGCTAT	402 GTCCAAAATAG	403 TTAATTGGCYA	404 GTCCAAAATRG
		TTTGGAC	CCAATTAA	TTTTGGAC	CCAATTAA
MDR1	G>A 70253 AC005068	405 TCTACTGGTATT	406 TAAGACAAATAC	407 TCTACTGGTRT	408 TAAGACAAAYAC
		TGTCTTA	CAGTAGA	TTGTCTTA	CAGTAGA
MDR1	C>A 70371 AC005068	409 AATCATTTTATG	410 TGTGGCACAIA	411 AATCATTTTMT	412 TGTGGCACAKA
		TGCCACA	AAATGATT	GTGCCACA	AAATGATT
MDR1	C>T 137 M29445	413 GAACATTGCITA	414 GTCTCCATAAG	415 GAACATTGCYT	416 GTCTCCATARG
		TGGAGAC	CAATGTTC	ATGGAGAC	CAATGTTC
MDR1	C>T 176 M29445	417 GAAGAGATIGT	418 CCCTCACAAATC	419 GAAGAGATYG	420 CCCTCACRATC

MDR1	A>C 43263 AC005068	GAGGG	TCCTC	TGAGGGC	TCCTC
		421 TGAATGTTCCG	422 CGGAGCCACGG	423 TGAATGTTCM	424 CGGAGCCACKG
		TGGCTCCG	AACATTCA	GTGGCTCCG	AACATTCA
MDR1	T>A 43162 AC005068	425 CGGGTGGTGAC	426 CTTCCTGTGICA	427 CGGGTGGTGW	428 CTTCCTGTGWC
		ACAGGAAG	CCACCCG	CACAGGAAG	ACCACCCG
MDR1	C>T 145984 AC002457	429 AAAATACTTIGG	430 CAAATTTCCAAA	431 AAAATACTTYG	432 CAAATTTCCRAA
		AAATTTG	GTATTTT	GAAATTTG	GTATTTT
MDR1	T>C 171404 AC002457	433 ATCATTAAACGA	434 ACTCATTTCGTT	435 ATCATTAAAYG	436 ACTCATTTCRTT
		AATGAGT	TAATGAT	AAATGAGT	TAATGAT
MDR1	G>C 171456 AC002457	437 GACTAAAGACA	438 CATTATGTGTC	439 GACTAAAGASA	440 CATTATGTSTC
		CATAAATG	TTTAGTC	CATAAATG	TTTAGTC
MDR1	G>T 171466 AC002457	441 GACATAAATGTT	442 AAACAACATAA	443 AGACATAAATG	444 AAACAACATA
		ATGTTTGT	CATTATGTCT	KTATGTTTGT	MCATTTATGTC
MDR1	T>C 171511 AC002457	445 GATACAGGGCT	446 TCATGAAGAGC	447 GATACAGGY	448 TCATGAAGARC
		CTTCATGA	CCTGTATC	TCTTCATGA	CCTGTATC
MDR1	T>C 171512 AC002457	449 GATACAGGGTC	450 ATTCATGAAGG	451 GATACAGGT	452 ATTCATGAAGR
		CTTCATGAAT	ACCCTGTATC	YCTTCATGAAT	ACCCTGTATC

MDR1	G>A	174901 AC002457	453 GTGCACGATAT TGGGGGAGC	454 GCTCCCCAAIA TCGTGCAC	455 GTGCACGATR TTGGGGAGC	456 GCTCCCCAAYA TCGTGCAC
MDR1	C>T	175068 AC002457	457 TAAGCAGCAAI AATGTCGTGT	458 ACACGACATTAT TGCTGCTTA	459 TAAGCAGCAA YAATGTCGTGT	460 ACACGACATTRT TGCTGCTTA
MDR1	C>T	175074 AC002457	461 CAAACAATGTIGT GTGCATC	462 GATGCACACAA CATTGTTG	463 CAAACAATGTYG TGTGCATC	464 GATGCACACRA CATTGTTG
MDR1	A>G	175142 AC002457	465 CATTAAATGGA GGACTGGG	466 CCCAGTCCTCC ATTTAATG	467 CATTAAATGRA GGACTGGG	468 CCCAGTCCTYC ATTTAATG
MDR1	A>G	175180 AC002457	469 TCCTCTGAGGA TGTGCAGT	470 ACTGCACATCC TCAGAGGA	471 TCCTCTGAGR ATGTGCAGT	472 ACTGCACATYCT CAGAGGA
MDR1	A>G	139015 AC002457	473 AACTTACTTGTA TCITTTGA	474 TCAAAGATACAA GTAAAGTT	475 AACTTACTTTR ATCTTTGA	476 TCAAAGATAYAA GTAAAGTT
MDR1	A>T	139064 AC002457	477 AGAAATAGTITA ATCAACA	478 TGTTGATTAAAC TATTTCT	479 AGAAATAGTW TAATCAACA	480 TGTTGATTAWA CTATTTCT
MDR1	T>C	139119 AC002457	481 TAGGGAGGGCT TAAGGCCA	482 TGGCCTTAAGC CCTCCCTA	483 TAGGGAGGGY TTAAGGCCA	484 TGGCCTTAARC CCTCCCTA
MDR1	G>A	139177 AC002457	485 GAAAGGTGAAA	486 TTGCTTTATITC	487 GAAAGGTGAR	488 TTGCTTTATYTC

MDR1	C>T	139276 AC002457	TAAAGCAA 489 CATTACCC <del>I</del> AG ATGGACC	ACCTTTC 490 GGTCATCT <del>A</del> G GGTAAATG	ATAAAGCAA 491 CATTACCC <del>Y</del> A GATGGACC	ACCTTTC 492 GGTCATCT <del>R</del> G GGTAAATG
MDR1	G>A	140118 AC002457	493 ATATGGAAG <del>A</del> A AATTACAA	494 TTGTAATTT <del>I</del> CT TCCATAT	495 ATATGGAAG <del>R</del> AAATTACAA	496 TTGTAATTT <del>Y</del> CT TCCATAT
MDR1	A>G	140216 AC002457	497 AACACGGG <del>C</del> GT TGATCTGA	498 TCAGATCAAC <del>G</del> CCCGTGTT	499 AACACGGG <del>R</del> TTGATCTGA	500 TCAGATCA <del>A</del> YG CCCGTGTT
MDR1	T>C	140490 AC002457	501 TGTATTAA <del>A</del> CGC GAATCCC	502 GGGATTGCG <del>G</del> T TTAATACA	503 TGTATTAA <del>A</del> YG CGAATCCC	504 GGGATTGCG <del>R</del> T TTAATACA
MDR1	G>A	140568 AC002457	505 TTGAAAGAC <del>A</del> T GTCTACAT	506 ATGTAGACA <del>T</del> G TCTTTCAA	507 TTGAAAGAC <del>R</del> T GTCTACAT	508 ATGTAGACA <del>A</del> YG TCTTTCAA
MDR1	A>T	140576 AC002457	509 CGTGTCTAC <del>T</del> TA AGTTGAA	510 TTCAACTTA <del>A</del> GT AGACACG	511 CGTGTCTAC <del>W</del> TAAGTTGAA	512 TTCAACTTA <del>W</del> G TAGACACG
MDR1	A>G	140595 AC002457	513 ATGTCCC <del>C</del> AGT GATTCAGC	514 GCTGAATCA <del>C</del> T GGGGACAT	515 ATGTCCC <del>C</del> CART GATTCAGC	516 GCTGAATCA <del>Y</del> T GGGGACAT
MDR1	G>A	140727 AC002457	517 CCGGGCCG <del>G</del> AA GCAGTCAT	518 ATGACTGCT <del>T</del> C CGGCCCGG	519 CCGGGCCG <del>R</del> AGCAGTCAT	520 ATGACTGCT <del>Y</del> C CGGCCCGG

MDR1	G>A 139479 AC002457	521 GAGGCGGGCA GATCACGAG	522 CTCGTGATCTG CCCGCCTC	523 GAGGCGGGCR GATCACGAG	524 CTCGTGATCYG CCCGCCTC
MDR1	T>C 139619 AC002457	525 GGAGAATGGCG TGAACCCG	526 CGGGTTCACGC CATTCTCC	527 GGAGAATGGY GTGAACCCG	528 CGGGTTCACRC CATTCTCC
MDR1	G>T 65241 AC005068	636 ACTAGAAAGGT CTGGGAAGGT	637 ACCTTCCCAGA ACCTTCTAGT	638 ACTAGAAAGGT KCTGGGAAGG	639 ACCTTCCCAGM ACCTTCTAGT
MDR1	G>A 50537 AC005068	640 TCCTGACTATAC CAAAGCCAA	641 TTGGCTTTGGI ATAGTCAGGA	642 TCCTGACTATR CCAAAGCCAA	643 TTGGCTTTGGY ATAGTCAGGA
TOP1	1334 133418 GI:11225259 G>T 45	529 ACTTTTCCGTIG CCGCGGCAACT	530 TTGCCGCGGCA ACGGAAAAGTT	531 ACTTTTCCGTK GCCGCGGCAA	532 TTGCCGCGGCM ACGGAAAAGTT
TOP1	1845 1845 GI:11225259 A>G	533 CTCGGGAAGGG CTCCATCAGA	534 TCTGATGGAGC CCTTCCCGAG	535 CTCGGGAAGG RCTCCATCAG	536 TCTGATGGAGY CCTTCCCGAG

Table 2: The nucleic acid and amino acid sequences referred to in this application

Gene	AS change	Protein Acc No	SEQ	Protein	SEQ	Protein wt>mut	ID N=	
							ID NO	ID N=
UGT1A1 L15R		G8850236	538	PLVLG <u>R</u> LLCVL	539	PLVLG <u>X</u> LLCVL		
UGT1A1 G71R		G8850236	540	LYIRD <u>R</u> A FYTL	541	LYIRD <u>X</u> A FYTL		
UGT1A1 D119Dframeshift		G8850236	542	KKIKK <u>D</u> CYA <u>F</u> C	543	KKIKK <u>D</u> X		
UGT1A1 P152Pframeshift		G8850236	544	VMLTDP <u>F</u> PS <u>L</u> Q	545	VMLTDP <u>X</u>		
UGT1A1 F170del		G8850236	546	LSLPTV <u>F</u> L <u>H</u> AL	547	LSLPTV <u>F</u> X		
UGT1A1 L175Q		G8850236	548	FFLHA <u>Q</u> PCSLE	549	FFLHA <u>X</u> PCSLE		
UGT1A1 C177R		G8850236	550	LHALP <u>R</u> SLEFE	551	LHALP <u>X</u> SLEFE		
UGT1A1 R209W		G8850236	552	MTFLQ <u>W</u> VKNML	553	MTFLQ <u>X</u> VKNML		
UGT1A1 P229Q		G8850236	554	DVVYS <u>Q</u> YATLA	555	DVVYS <u>X</u> YATLA		
UGT1A1 G276R		G8850236	556	NMVFV <u>R</u> GINCL	557	NMVFV <u>X</u> GINCL		

UGT1A1 A292V	G8850236	558	SQEFEVYINAS	559	SQEFEXYNAS
UGT1A1 Y293Wframeshift	G8850236	560	QEFEAWRTWN	561	QEFEAXINASG
UGT1A1 G308E	G8850236	562	VVFSLES MVSE	563	VVFSLXSMVSE
UGT1A1 Q331R	G8850236	564	LGKIPRTVLWR	565	LGKIPXTVLWR
UGT1A1 Q357R	G8850236	566	VKWLP <del>R</del> NDLLG	567	VKWLPXNDLLG
UGT1A1 R367G	G8850236	568	GHPMTGAFITH	569	GHPMTXAFITH
UGT1A1 A368T	G8850236	570	HPMTRIFITHA	571	HPMTRXFITHA
UGT1A1 P387R	G8850236	572	ICNGVRMVMMP	573	ICNGVXMVMMP
UGT1A1 S375F	G8850236	574	ITHAGFHGVYE	575	ITHAGXHG VYE
UGT1A1 S381R	G8850236	576	HGVYERICNGV	577	HGVYEXICNGV
UGT1A1 A401P	G8850236	578	DQMDNPKRMET	579	DQMDNXKRMET
UGT1A1 R403Rframeshift	G8850236	580	MDNAK <del>RHGD</del>	581	MDNAKX
UGT1A1 K428E	G8850236	582	LENAL <del>E</del> AVIND	583	LENALXAVIND

UGT1A1 Y486D	G8850236	584	LTWYQD <del>H</del> SLDV	585	LTWYQX <del>H</del> SLDV
UGT1A1 S488F	G8850236	586	WYQYH <del>F</del> LDVIG	587	WYQYH <del>X</del> LDVIG
UGT1A1 Q49stop	G8850236	588	LGAIQ <del>2</del>	589	LGAIQ <del>2</del>
UGT1A1 C280stop	G8850236	590	VGGIN <del>2</del>	591	VGGIN <del>2</del>
UGT1A1 Q331stop	G8850236	592	LGKIP <del>2</del>	593	LGKIP <del>2</del>
UGT1A1 W335stop	G8850236	594	PQTVL <del>2</del>	595	PQTVL <del>2</del>
UGT1A1 Q357stop	G8850236	596	VKWLP <del>2</del>	597	VKWLP <del>2</del>
UGT1A1 K437stop	G8850236	598	NDKSY <del>2</del>	599	NDKSY <del>2</del>
MRP1 F329C	G2828206	600	YFLMS <del>C</del> FFKAI	601	YFLMS <del>X</del> FFKAI
MRP1 R433S	G2828206	602	SVDAQ <del>S</del> FMDLA	603	SVDAQ <del>X</del> FMDLA
MRP1 R723Q	G2828206	604	QNDSL <del>Q</del> ENILF	605	QNDSL <del>X</del> ENILF
MDR1 N21D	G2506118	606	FFKLND <del>K</del> SEKD	607	FFKLND <del>X</del> SEKD
MDR1 F103L	G2506118	608	INDTGL <del>F</del> FMNLE	609	INDTGL <del>X</del> FMNLE



MDR1	V168I	G2506118	610	FDVHDIGELNT	611	FDVHD <del>X</del> GELNT
MDR1	S400N	G2506118	612	RNVHFN <del>Y</del> PSRK	613	RNVHF <del>X</del> YPSRK
MDR1	G412G	G2506118	614	VKILK <del>G</del> LN <del>L</del> KV	615	VKILK <del>X</del> LN <del>L</del> KV
MDR1	T436T	G2506118	616	CGKST <del>T</del> VQLMQ	617	CGKST <del>X</del> VQLMQ
MDR1	A893S	G2506118	618	KELEG <del>S</del> GKIAT	619	KELEG <del>X</del> GKIAT
MDR1	A999T	G2506118	620	FAPDY <del>T</del> KAKIS	621	FAPDY <del>X</del> KAKIS
MDR1	A1001T	G2506118	622	PDYAK <del>T</del> KISAA	623	PDYAK <del>X</del> KISAA
MDR1	Q1107P	G2506118	624	KRLNV <del>P</del> WLRAH	625	KRLNV <del>X</del> WLRAH
MDR1	A1132A	G2506118	626	IAENI <del>A</del> YGDNS	627	IAENI <del>X</del> YGDNS
MDR1	S1141T	G2506118	628	NSRVV <del>T</del> QEEIV	629	NSRVV <del>X</del> QEEIV
MDR1	I1145I	G2506118	630	VSQEEI <del>V</del> RAAK	631	VSQEE <del>X</del> VRAAK
TOP1	G363C	G12644118	632	PGLFR <del>C</del> RGNHHP	633	PGLFR <del>X</del> RGNHHP
TOP1	D533G	G12644118	634	DFLGK <del>G</del> SIRYY	635	DFLGK <del>X</del> SIRYY

**Table 3:** Selected nucleic acid sequences referred to in this application

Gene	Variation	SNP	Genbank Accession No	SEQ ID NO
UGT1A1	C>T	890	GI:8850235	037
UGT1A1	G>A	1117	GI:8850235	069
UGT1A1	T>G	1471	GI:8850235	097
Cyp3A5	T>C	47518	GI:10281451	137
Cyp3A5	T>G	145601	GI:11177452	141
Cyp3A5	A>G	145929	GI:11177452	145
Cyp3A5	A>G	9736	GI:10281451	149
MRP1	C>T	137647	AC026452	181
MRP1	T>C	95	AF022831	209
MRP1	C>G	53282	GI:7209451	217
MRP1	T>G	249	AF022830	205
MRP1	A>G	259	AF022831	277
MRP1	T>C	124667	AC026452	281
MRP1	A>G	381	U07050	301
MRP1	<i>inst</i>	926/927	U07050	325
MRP1	G>A	34218	AC003026	229
MRP1	C>T	18067	U91318	193
MRP1	C>T	440	U07050	313
MRP1	C>A	1625	U07050	293
MRP1	C>T	17900	U91318	253
MDR1	G>A	101	M29432	345
MDR1	C>T	176	M29445	417
MDR1	G>T	88883	GI:10122135	636

**Table 4:** Selected amino acid sequences referred to in this application

Gene	AA change	Protein Genbank No	SEQ ID NO
UGT1A1	A292V	G8850236	558
UGT1A1	A368T	G8850236	570
UGT1A1	Y486D	G8850236	584
MRP1	F329C	G2828206	600
MDR1	S400N	G2506118	612
MDR1	A893S	G2506118	618

The figure show:

Figure 1 shows the correlation of the exon 26 SNP with intestinal MDR1 expression in 21 volunteers determined by Western blot analyses. The box plot shows the distribution of MDR1 expression clustered according to the MDR1 3435C>T genotype at position corresponding to position 176 of the MDR1 gene (GenBank Acc. No. M29445). The T allele was associated with a lower expression of p-glycoprotein.

Figure 2 shows the correlation of MDR1 3435C>T genotype and digoxin uptake in 14 healthy volunteers who participated in a clinical study that addresses peak plasma levels of digoxin at steady state [Johns et al., 1999, Clin. Pharmacol. Ther 66:338-345]. Maximum digoxin levels were statistically significantly different ( $p=0.006$ , Mann Whitney U test) between the two groups which were homozygous for the T and C allele, respectively.

Figure 3 represent the correlation of the genotype (wt/wt: 1; wt/mut and mut/mut:2) with MRP1 mRNA content in duodenal biopsies from healthy volunteers derived from two independent experiments, before and after application of rifampicin. Treatment with rifampicin had no effect on MRP1 mRNA expression ( $p<0.001$ , paired t-test). A strong trend of an association of MRP1 genotype with MRP1 mRNA levels was detected ( $p=0.086$ , Kruskal-Wallis test).

Figures 4 to 28 show the nucleic acid and amino acid sequences referred to herein.

Figure 29 shows the expression profile of genes relevant to Irinotecan metabolism in carcinoma cell lines. This semiquantitative RT-PCR shows amounts of transcripts for the genes indicated right to the amplicons. PCR products were analyzed by agarose electrophoresis, stained with ethidium bromide. The respective fragment sizes are indicated on the left in basepairs (bp).

Figure 30 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with epithelial carcinoma cell lines LS174T (colon), KB 3-1 (cervix) and RT112 (bladder). Concentrations of CPT-11 ranged from 0 to 200  $\mu\text{g/ml}$  and of SN-38 from 0 to 200 ng/ml. Cells were treated for three days. The data for each concentration are mean values of at least three wells.

Figure 31 growth inhibition curves for CPT-11 (A) and SN-38 (B) with a epithelial cervix carcinoma cell line KB 3-1 and two subclones expressing high amounts of MDR1, KB 3-1 (MDR1) and KB 3-1 (MDR1, CYP3A5). Concentrations of CPT-11 ranged from 0 to 200  $\mu\text{g/ml}$  and of SN-38 from 0 to 200 ng/ml. Cells were treated for three days. The data for each concentration are mean values and standard deviation of at least three wells.

Figure 32 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with the bladdercancer cell line RT112 and and its subclones RT112 (MDR1, UGT1A1) expressing MDR1 and higher amounts of UGT1A1. Concentrations of CPT-11 ranged from 0 to 200  $\mu\text{g/ml}$  and of SN-38 from 0 to 200 ng/ml. Cells were treated for three days. The data for each concentration are mean values and standard deviation of at least three wells.

Figure 33 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with inhibition of MDR1 by R-Verapamil. The epithelial cervix carcinoma cell line KB 3-1 and the two subclones KB 3-1 (MDR1) and KB 3-1 (MDR1, CYP3A5), with high MDR1 expression, were tested for the influence of MDR1 inhibition by R-Verapamil on drug sensitivity. Concentrations of CPT-11 ranged from 0 to 200  $\mu\text{g/ml}$  and of SN-38 from 0 to 200 ng/ml and R-Verapamil was added to 10  $\mu\text{g/ml}$  final concentration(+V). Cells were treated for three days. The data for each concentration are mean values of two wells.

Figure 34 shows growth inhibition curves for CPT-11 (A) and SN-38 (B) with inhibition of MDR1 by R-Verapamil. To circumvent the MDR1 effect on drug resistance cells were treated in parallel with R-Verapamil. The KB 3-1 (MDR1) and KB 3-1 (MDR1, CYP3A5), which differ in their CYP3A5 expression, were tested for remaining resistance after inhibition of MDR1. Concentrations of CPT-11 ranged from 0 to 200  $\mu$ g/ml and of SN-38 from 0 to 200 ng/ml and R-Verapamil was added to 10  $\mu$ g/ml final concentration(+V). Cells were treated for three days. The data for each concentration are mean values of two wells.

The present invention is illustrated by reference to the following biological Examples which are merely illustrative and are not to be constructed as a limitation of the scope of the present invention.

**Example 1: Phenotypically impact of the C to T substitution at position corresponding to position 176 of the MDR1 gene (Acc. No. M29445).**

To investigate the influence of the single nucleotide C to T substitution at position corresponding to position 176 of the MDR1 gene (Acc. No. M29445) also referred to as MDR1 exon 26 SNP C3435T on intestinal P-glycoprotein (PGP) expression, samples from biopsies and duodenal enterocyte preparations from 21 were investigated at the Dr. Margarete Fischer-Bosch-Institute for Clinical Pharmacology in Stuttgart by quantitative immunohistochemistry and Western blots. The results are shown in Figure 1. Homozygous carriers of the T allele (having at a position corresponding to position 176 of the MDR1 gene (Accession No: M29445) a T) demonstrated significantly higher PGP levels compared to homozygous carriers of the C allele (having at a position corresponding to position 176 of the MDR1 gene (Accession No: M29445) a C). Individuals with heterozygous genotype showed an intermediate level of PGP expression.

Furthermore, the influence of the MDR1 genotype on intestinal uptake-related pharmacokinetics of digoxin was investigated in a clinical study at the University Medical Center, Charite in Berlin. Maximal digoxin blood levels ( $C_{max}$ ) at steady state were correlated with the MDR1 3435C>T genotype 14 healthy volunteers after

oral application of digoxin. Figure 2 shows, volunteers homozygous for the T allele show statistically significantly lower digoxin levels than volunteers with a C/C genotype. ( $p=0.006$ , Mann Whitney U test) and reflects the impact of this polymorphism on digoxin pharmacokinetics.

### **Example 2: Correlation of MRP1 polymorphisms with MRP1 expression and side effects during therapy with MRP1 substrates**

Functional polymorphisms in the MRP1 gene affect the transport activity which in consequence modulates plasma levels and/or intracellular concentrations of MRP1 substrate drugs. Increased levels of such drugs can lead to side effects whereas decreased levels may result in subtherapeutical drug levels and therapy failure. MRP1 polymorphisms were correlated with the occurrence of drug-related adverse effects and therapeutic efficacy in patients treated with MRP1 substrate drugs. In a case-control study, the frequency distribution of MRP1 SNPs was compared between a group of patients who suffered from cisplatin-related nephrotoxicity and a group of patients with nephro- and hepatotoxicities caused from anti-cancer drugs with a group of healthy controls. Furthermore, samples of known MRP1 mRNA levels were screened for MRP1 genotype. The results in the group of patients demonstrating nephro- and hepatotoxicity during anti-cancer treatment, are listed in the following table for one MRP1 SNP:

SNP	group	Allele frequency [%]		Genotype frequency [%]		
		G allele	A allele	*G/A	*A/A	*A/A expected <sup>2</sup>
150727G>A <sup>1</sup>	Controls	66.7	33.3	50	8.3	10.9
	Cases	50.0	50.0	14.3	42.9	25.0

<sup>1</sup>according to Acc. No. AC025277

<sup>2</sup>calculated according to Hardy-Weinberg

In contrast to control samples, the A allele (substitution of G to A at position according to position 150727 of the MRP1 gene, Acc. No. AC025277) was

statistically significantly overrepresented in patients suffering from drug-related kidney- and liver side effects compared to healthy controls ( $p=0.044$ ,  $\chi^2$  test) and was thus predictive for these side effects.

Furthermore, an association of MRP1 genotype with mRNA expression before and after rifampicin application was detected for two MRP1 SNP's, 95T>C (SEQ ID NOs. 209, 210, 211, and 212, nucleotide substitution of T to C at a position corresponding to position 95 of the MRP1 gene, Acc. No. AF022831) and 259A>G (SEQ ID NOs. 277, 278, 279, and 280, nucleotide substitution of A to G at a position corresponding to position 259 of the MRP1 gene, Acc. No. AF022831). These SNPs are linked and form one allele. The mutant allele (MRP1mut, C at position 95 and G at position 259 of the MRP1 gene, Acc. No. AF022831) is statistically significantly correlated with decreased MRP1 mRNA expression and the wildtype allele (MRP1wt, T at position 95 and A at position 259 of the MRP1 gene, Acc. No. AF022831) with increased MRP1 expression in two independent experiments (with and without rifampicin induction), as illustrated in figure 3.

The differences in the MRP1 mRNA content are based on MRP1 genotype-related interindividual differences and the analysis of these SNP's is of high diagnostic and prognostic value for MRP1 expression levels and to predict the therapeutic outcome and adverse effects of MRP1 substrate drugs.

### **Example 3: Dosage calculation**

Therapeutic efficacy and adverse effects of irinotecan depend on plasma levels and intracellular concentrations of the parent compound and the active metabolites (e.g. SN-38), processes which are controlled by CYP3A5- and UGT1A1-related metabolism and MRP1- and MDR1-related transport processes [Atsumi, *et al.*, 1991, *Xenobiotica* 21:1159-69, Iyer, *et al.*, 1998, *J Clin Invest* 101:847-54, Ciotti, *et al.*, 1999, *Biochem Biophys Res Commun* 260:199-202, Santos, *et al.*, 2000, *Clin Cancer Res* 6:2012-20, Kuhn, 1998, *Oncology (Huntingt)* 12:39-42, Chen, *et al.*, 1999, *Mol Pharmacol* 55:921-8, Chu, *et al.*, 1997, *Cancer Res* 57:1934-8, Chu, *et al.*, 1997, *J Pharmacol Exp Ther* 281:304-14; Chu, *et al.*, 1998, *Cancer Res* 58:5137-43, Chu, *et al.*, 1999, *Drug Metab Dispos* 27:440-1, Chu, *et al.*, 1999, *J Pharmacol Exp Ther* 288:735-41, Mattern, *et al.*, 1993, *Oncol Res* 5:467-74, Hoki,



*et al.*, 1997, *Cancer Chemother Pharmacol* 40:433-8, Sugiyama, *et al.*, 1998, *Cancer Chemother Pharmacol* 42:S44-9]. For example, MRP1 works in close connection with glucuronosyltransferases as part of the cellular detoxification system and is known to transport glucuronosyl conjugates such as SN-38G [König *et al.*, 1999, *Biochim Biophys Acta* 1461:377-394, Kerb *et al.*, 2001, *Pharmacogenomics* 2:51-64]. For example, the extent to which SN-38G is exported from the cell into bile greatly influences the rate of its formation. For an efficient detoxification of SN-38 both processes are necessary, conjugation by UGT1A1 and export of the glucuronide.

The 47523T>C (SEQ ID NOs. 137, 138, 139, and 140) and 35649A>G (SEQ ID NOs. 149, 150, 151, 152) nucleotide substitutions of the CYP3A5 gene (Acc. No. GI:10281451), and the 145601T>G (SEQ ID NOs. 141, 142, 143, 144) and 145929A>G (SEQ ID NOs. 145, 146, 147, and 148) nucleotide substitutions of the CYP3A5 gene (Acc. No. GI:11177452) form an high CYP3A5 expression-related allele and are therefore associated with a higher metabolic inactivation of irinotecan. Individuals with this allele are extensive metabolizers (EMs) and are therefore in contrast the remainder poor metabolizers (PMs) less likely to suffer from irinotecan toxicity. Those with one high expressor and one low expressor-related allele are regarded as intermediate metabolizers (IMs).

The 176C>T nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MDR1 gene (Accession No: M29445) is associated with low PGP expression-related low drug efflux, and the 95T>C (SEQ ID NOs. 209, 210, 211, and 212) and the 259A>G (SEQ ID NOs. 277, 278, 279, and 280) nucleotide substitutions of the MRP1 gene (Acc. No. AF022831) are associated with low mRNA expression and the 150727G>A nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MRP1 gene (Accession No: M29445) is associated with low PGP expression-related low drug efflux and the 150727G>A nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MRP1 gene (Accession No: AC025277) is associated with adverse effects. Individuals carrying low transporter expression-related alleles are therefore less capable to clear cells from toxic compounds. Both, transport and metabolism are affected in a gene-dose dependant manner. According to the number of low expression-related alleles of the respective transport protein, individuals can be classified as having either extensive (ET), intermediate (IT) or poor transporter capacity (PT) of the respective gene.

By genetic testing prior to onset of treatment with irinotecan, the MDR1- and MRP1-related transport capacity of the patients can be predicted. The individual risk to adverse effects depends on the number of PM and/or PT alleles. Individuals with PM-related alleles of CYP3A5 and UGT1A1 and PT-related alleles of MDR1 and MRP1 are at the highest risk to suffer from irinotecan toxicity.

Based on this knowledge, the initial dose can be adjusted prior to the first dose as shown by Brockmüller et al. (2000, *Pharmacogenomics* 1:125) for substrate drugs of CYP2D6, CYP2C9, and CYP2C19.

Dose adjustment can be achieved using a scoring system. For each PM- or PT-related allele a certain score is assigned e.g. a score of 2 is assigned to UGT1A1 PM alleles 226A, (SEQ ID NOs 9, 10, 11, 12, 540, 541) and 701A (SEQ ID NOs. 25, 26, 27, 28, 554, 555), and a score of 1 is assigned to the CYP3A5 PM-related alleles (47523T plus 35649A plus 145601T plus 145929A, 47523T plus 35649G plus 145601G plus 145929G, and 47523C plus 35649A plus 145601T plus 145929A), to the MDR1 low expression allele 176T (SEQ ID NOs.: 417, 418, 419, and 420), to the MRP1 low expression alleles 150727A (SEQ ID NOs. 217, 218, 219, and 220) and 259G (SEQ ID NOs. 277, 278, 279, and 280), to the MRP1 150727A allele (SEQ ID NOs. 217, 218, 219, and 220). After genotyping the scores are summarized and irinotecan dosage is adjusted according to the sum. Each single score corresponds to a dose reduction of 10%, i.e. a score of one corresponds to a 10% dose reduction, a score of two to 20%, a score of 3 to 30%, etc.

#### **Example 4: Culture conditions and biological assays**

The human epithelial cervical cancer cell line KB 3-1 with two subclones (KB 3-1 (MDR1<sup>+++</sup>) and KB 3-1 (MDR1<sup>+++</sup>, CYP3A5)) and the bladder cancer cell line RT112, also with subclone (RT112 (MDR1<sup>+</sup>, UGT1A1)), were cultured in Dulbecco's Modified Eagle Medium (DMEM) including 3.7 g/l NaHCO<sub>3</sub>, 4.5 g/l D-Glucose, 1.028 g/l N-Acetyl-L-Alanyl-L-glutamine and supplemented with 10% fetal bovine, 1 mM Na-pyruvate and 1% non-essential amino acids. The human colon cancer cell line LS174T was cultured in Dulbecco's modified Eagle medium containing L-glutamine, pyridoxine hydrochloride and 25 mM Hepes buffer without phenol red, supplemented with 10% fetal bovine, 1 mM Na-pyruvate and 1% non-essential

amino acids. All cells were incubated at 37°C with 5% CO<sub>2</sub> in a humidified atmosphere.

### **Drugs**

Irinotecan (CPT-11) and its active metabolite SN-38 were provided by Pharmacia. For preparation of stock solutions the substances were dissolved in methanol, 10 mg/ml for CPT-11 and 1 mg/ml for SN-38 and stored at 4°C protected from light. Lower concentrated dilutions were prepared in PBS and cell culture medium. R-Verapamil was applied from SIGMA, dissolved in DMSO to 50 mg/ml and further diluted in PBS.

### **Treatment of cells with drugs**

Cells were seeded in 96-well culture plates 24 h prior to treatment. With respect to differential growth rates KB 3-1 and RT112 cells were seeded at 700 cells/well, RT112 (MDR1<sup>+</sup>, UGT1A1) at 1000 cells/well and KB 3-1 (MDR1<sup>+++</sup>) and KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) at 1200 cells/well. LS174T were seeded at 1.0 x 10<sup>4</sup> cells/well. Cells were treated with freshly prepared serial dilutions in culture medium, 0, 0.5, 1, 2.5, 5, 7.5, 10, 25, 50, 75, 100 and 200 µg/ml for CPT-11, and 0, 0.1, 0.25, 0.5, 1, 5, 10, 25, 50, 75, 100 and 200 ng/ml for SN-38. Four well were treated with the same drug dilution. Cells were incubated for 3 days at 37°C in a humidified 5% CO<sub>2</sub> atmosphere.

For MDR1 inhibition experiments R-Verapamil was added to 10 µg/ml final concentration in two wells of each drug dilution.

### **Cytotoxicity assay**

A commercially available MTS assay system (Promega, Madison, USA) was used to determine growth inhibition and cell death according to the instructions of the manufacturer. Three days after adding the drugs, 20 µl of the combined MTS/PMS solution was added to each well of the 96-well culture plate. The plate was incubated for at least 45 min at 37°C in a humidified 5% CO<sub>2</sub> atmosphere and the absorbance at 492 nm was measured. The absorbance values of untreated control

cells on each plate were set as 100% growth and used to calculate the remaining growth of drug treated cells. Untreated cells on the culture plates served as controls for unaffected growth and survival.

The drug concentration effecting a 50% inhibition of cell growth was defined as the  $IC_{50}$ .

### **RNA preparation and cDNA synthesis**

From each cell batch used in these experiments messenger RNA was isolated from cell lysates by oligo-dT magnet beads ( $\mu$ MACS mRNA Isolation Kit; Miltenyi Biotech) following the instructions of the manufacturer. 250 ng mRNA of each cell line was applied in a 20  $\mu$ l cDNA synthesis reaction with Superscript II reverse transcriptase (Gibco BRL). Dilutions of this cDNAs served as template in transcript specific amplification reactions.

### **PCR primers and reaction conditions**

PCRs were set up in 25  $\mu$ l reactions with 0.5 units Taq Polymerase (Qiagen), 200  $\mu$ M nucleotide mix, 5  $\mu$ l cDNA template dilution and 0.2  $\mu$ M gene specific primers, as indicated in Table 5. All reactions were run under the same amplification conditions, differing only in number of cycles (table ), 2 min pre-denaturation at 94°C, than for amplification: 45 sec denaturation at 94°C, 45 sec annealing at 62°C and 45 sec elongation at 72°C, except for UGT1A1 which needed longer elongation of 2 min.

**Table 5:** Sequences of gene specific primers and conditions for PCR reactions. F: forward primer; R: reverse primer for mRNA sequences.

Gene	Primer sequence (5'-3')	cDNA dilution	cycle number
MDR1	F: TGCCTTCATCGAGTCACTGCC R: TCACTGGCGCTTTGTTCCAGC	1:100	26
MRP1	F: TCTCCAAGGAGCTGGACACA R: CGTGGTGACCTGCAATGAGT	1:10	30
UGT1A	F: GATGATGCCCTTGTTTGGTG R: TGTTTTCAAGTTTGGAATGACTAGGG	1:100	30
UGT1A1	F: AACCTCTGGCAGGAGCAAAGG R: TGTTTTCAAGTTTGGAATGACTAGGG	1:10	34
CYP3A4	F: TCAGCCTGGTGCTCCTCTATCTAT R: AAGCCCTTATGGTAGGACAAAATATTT	1:10	34
CYP3A5	F: TTGTTGGGAAATGTTTTGTCCTATC R: ACAGGGAGTTGACCTTCATACGTT	1:10	34
PLA2 (house keeping gene)	F: GCTGGTTCAGAAGGCCAAAC R: GGGCCAGACCCAGTCTGATA	1:100	26

#### Example 5: Expression of genes involved in irinotecan metabolism

Messenger RNA was isolated from the human bladder cancer cell line RT112, its subclone RT112 (MDR1, UGT1A1), the human epithelial cervical cancer cell line KB 3-1 and two subclones KB 3-1 (MDR1<sup>+++</sup>) and KB 3-1 (MDR1<sup>+++</sup>, CYP3A5), and the colon carcinoma cell line LS174T (ATCC CL-188). These mRNAs were reverse transcribed into cDNA and applied as templates in transcript-specific amplification reactions to determine the expression levels of genes involved in irinotecan transport and metabolism (MDR1, MRP1, UGT1A, UGT1A1, CYP3A4, CYP3A5).

Amplification of the house keeping gene phospholipase A2 (PLA2) was used as a control for comparable cDNA amounts in the reactions.

The amplification reactions in figure 29 show that the carcinoma cell lines RT112, KB 3-1, and LS174T have no or very low expression of MDR1, respectively. RT112 (MDR1, UGT1A1) is a subclone of RT112, which was selected for resistance to cytotoxic drugs as described in Seemann et al. (Urol Res 1995; 22:353-360), and is characterised by a moderately increased MDR1 expression. The drug resistant subclones KB 3-1 (MDR1<sup>+++</sup>) and KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) were derived similarly from the original KB 3-1 cell line by exposure to MDR1 substrates. These subclones are characterized by highly increased MDR1 expression. They show >20-times more transcripts than the original KB 3-1 cells, implicating a very high MDR1 activity. MRP1 is expressed at the same level in all cell lines. Transcripts of UGT1A enzymes are present only in RT112, RT112 (MDR1, UGT1A1), and LS174T cells. UGT1A1 is only weakly expressed in RT112, stronger expressed in RT112 (MDR1, UGT1A1) and shows highest expression in LS174T cells. CYP3A4 was solely detected in very small amounts in LS174T. RT112 cells, RT112 (MDR1, UGT1A1), and LS174T show a heterozygous expression of the functionally inactive splice variant and the functionally active transcript of CYP3A5. In contrast, KB 3-1 and KB 3-1 (MDR1<sup>+++</sup>) cells have only the active CYP3A5 transcript and the KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) showed the highest expression of the active CYP3A5 transcript, implicating that the latter have the highest CYP3A5 activity.

**Example 6: Colon and other epidermal cancer cell lines with no or low MDR1 and CYP3A5 activity are sensitive to CPT-11 and SN-38.**

The colon cancer cell line LS174T, the cervical cancer cell line KB 3-1 and the bladder cancer cell line RT112 were seeded in 96-well culture plates 24 h prior to treatment. Four wells of each cell line were incubated with serial dilutions of CPT-11 and SN-38 and analysed as described above. Figure 30 shows that all three epidermal cancer cell lines stop proliferation and die upon treatment with CPT-11 and SN-38. The concentrations resulting in 50% inhibition (IC<sub>50</sub>) for CPT-11 are 1.5 µg/ml for LS174T, 2.5 µg/ml for RT112 and 5 µg/ml for KB 3-1 cells. The active metabolite of CPT-11, SN-38 shows a 1000-fold higher efficacy than CPT-11, since

$10^3$ -times lower concentrations cause the same degree of growth inhibition and cell death. The  $IC_{50}$  of SN-38 is 5 ng/ml for LS174T cells, 4 ng/ml for RT112 cells and 25 ng/ml for KB 3-1 cells.

These results show that all three epidermal cancer cell lines although derived from different tissues are similarly sensitive to CPT-11 and SN-38 treatment. This also indicates that cancer cells expressing no or only low levels of MDR1 (Figure 29) can be efficiently killed by CPT-11 and SN-38 (Figure 30).

#### **Example 7: MDR1 activity correlates with resistance of cancer cells toward CPT-11 and SN-38**

Cells of KB 3-1 and its strongly MDR1 expressing subclones KB 3-1 (MDR1<sup>+++</sup>) and the KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) were seeded in 96-well culture 24 h prior to treatment. Four wells of each cell line were incubated with serial dilutions of CPT-11 and SN-38 and treated as described above. The inhibition curves (Figure 31) of the MDR1 high expresser KB 3-1 subclones (KB 3-1 (MDR1<sup>+++</sup>) and KB 3-1 (MDR1<sup>+++</sup>, CYP3A5)) (Figure 29) demonstrate a significant higher resistance to CPT-11 and SN-38 compared to the MDR1 low expresser KB 3-1 cell line (KB 3-1). The  $IC_{50}$  for CPT-11 increases 17 to 40 fold from 5  $\mu$ g/ml in KB 3-1 to 85  $\mu$ g/ml in KB 3-1 (MDR1<sup>+++</sup>) and 200  $\mu$ g/ml in KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) cells. The  $IC_{50}$  for SN-38 increases at least 8 times from 25 ng/ml in KB 3-1 to 200 ng/ml in KB 3-1 (MDR1<sup>+++</sup>) and  $\geq 200$  ng/ml in KB 3-1 (MDR1<sup>+++</sup>, CYP3A5).

CPT-11 and SN-38 are substrates of MDR1, and are therefore removed from the cells by MDR1 activity. The MDR1 expression level correlates inversely with the sensitivity of tumor cells towards CPT-11 and SN-38. Subsequently, the killing of cells with high MDR1 expresser phenotype requires much higher concentrations of CPT-11.

**Example 8: UGT1A1 activity correlates with sensitivity towards SN-38 and not towards CPT-11**

CPT-11 and SN-38 sensitivity was compared between RT112 cells and its subclone RT112 (MDR1, UGT1A1). Four wells of each cell line were incubated with serial dilutions of CPT-11 and SN-38 and treated as described above.

The difference in sensitivity against CPT-11 is only small as shown in Figure 32A. The  $IC_{50}$  of RT112(MDR1, UGT1A1) cells of 4  $\mu$ g/ml CPT-11 is two-times higher compared to RT112 cells ( $IC_{50}$  of 2.5  $\mu$ g/ml). In contrast to RT112 cells which express no MDR1, RT112 MDR1, UGT1A1) cells express an intermediate amount of MDR1 which can explain the small though significant increase of CPT-11 sensitivity. A much stronger difference exists between RT112 ( $IC_{50}$  of 4 ng/ml) and RT112 (MDR1, UGT1A1) cells ( $IC_{50}$  of 75 ng/ml) after treatment with SN-38 (Figure 32B). This 19-fold higher resistance of the RT112 (MDR1, UGT1A1) cell line can be explained by the additional detoxifying effect of UGT1A1 which is expressed at a higher level in RT112 (MDR1, UGT1A1) than in RT112 cells (Figure 29). In contrast to SN-38, CPT-11 is not metabolized by UGTs. Therefore, CPT-11-related toxicity is not affected by UGT1A1 expression and the resistance-enhancing capability of UGTs in RT112(MDR1, UGT1A1) cells is only detected by application of SN-38.

**Example 9: MDR1 inhibition serves as sensitizer towards CPT-11 and SN-38 in MDR1 high expressing but not low expressing cancer cells.**

The sensitivity of KB 3-1 cells and its subclones KB 3-1 (MDR1<sup>+++</sup>) and KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) against CPT-11 and SN-38 was assessed after blocking MDR1 function using the specific inhibitor R-Verapamil. Four wells of each cell line were incubated with serial dilutions of CPT-11, SN-38 and analysed as described above. Two wells were additionally treated with the MDR1 inhibitor R-Verapamil. Figure 33 shows that addition of R-Verapamil has only marginal effects on the CPT-11 and SN-38 sensitivity of MDR1 low expresser KB 3-1 cells (CPT-11 and SN-38  $IC_{50}$ s of 5  $\mu$ g/ml and 25 ng/ml without R-Verapamil versus 4.5  $\mu$ g/ml and 15 ng/ml with R-Verapamil, respectively). In contrast, the sensitivity of the MDR1 expressing cells KB 3-1(MDR1<sup>+++</sup>) and KB 3-1(MDR1<sup>+++</sup>, CYP3A5) towards CPT-11 and SN-38



was 8-fold and 10-fold higher after inhibition of MDR1 transport function with R-Verapamil. The  $IC_{50}$  of KB 3-1(MDR1<sup>+++</sup>) cells for CPT-11 decreased from 85  $\mu$ g/ml without to 10  $\mu$ g/ml with R-Verapamil and from 200  $\mu$ g/ml without to 25  $\mu$ g/ml with R-Verapamil in KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) cells. The effect of MDR1 inhibition during SN-38 treatment is even stronger in these MDR1 high expresser cells, R-Verapamil blocked the MDR1 transport completely and they become as sensitive as KB 3-1 cells.

These results demonstrate that the MDR1 activity is relevant for resistance of cancer cells to CPT-11 and SN-38 and that inhibition of MDR1 sensitises the cells, so that they are more efficiently killed at lower drug concentrations.

#### **Example 10: CYP3A5 activity influences resistance to CPT-11**

KB 3-1 (MDR1<sup>+++</sup>) and KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) cells which differ by their amounts of CYP3A5 (Figure 29). Four wells of each cell line were incubated with serial dilutions of CPT-11, SN-38 and analyzed as described above. Two wells were additionally treated with the MDR1 inhibitor R-Verapamil.

Because MDR1 activity is a major determinant of cellular sensitivity toward CPT11 and SN-38, the MDR1 activity in these MDR1 high expresser cell lines was completely blocked using an excess of the specific MDR1 inhibitor R-Verapamil to analyze the impact of CYP3A5 on CPT-11 and SN-38 sensitivity without interference of MDR1.

The high CYP3A5 expresser cell line KB 3-1 (MDR1<sup>+++</sup>, CYP3A5) is with an  $IC_{50}$  of 25  $\mu$ g/ml 2.5-times more resistant to CPT-11 than KB 3-1 (MDR1<sup>+++</sup>) showing an  $IC_{50}$  of 10  $\mu$ g/ml (Figure 34). No difference between these two cell lines can be observed regarding their sensitivity towards SN-38.

These experiments demonstrate a significant impact of CYP3A5 expression on the resistance to CPT-11 in contrast to SN-38. The fact that CYP3A5 activity had no

influence on SN-38 toxicity further confirms the CYP3A5 effect, because CPT-11 but not SN-38 is metabolized by CYP3A5.

**Example 11: MDR1 genotyping improves therapeutic efficacy of irinotecan by genotype-based prediction and monitoring of drug resistance.**

Therapeutic efficacy and adverse effects of irinotecan depend on plasma levels and on intracellular tumor concentrations of the parent compound and the active metabolites (e.g. SN-38). The MDR1 gene controls the PGP-dependent penetration of irinotecan across membranes [Luo et al., Drug Metab Dispos 2002, 30:763-770; Jansen et al., Br J Cancer 1998, 77:359-65 Chu et al., J Pharmacol Exp Ther 1999; 288, 735-41; Sugiyama et al., Cancer Chemother Pharmacol 1998, 42 Suppl:S44-9] and is therefore an important determinant for its systemic availability and intracellular accumulation. The 176C>T nucleotide substitution (SEQ ID NOs. 217, 218, 219, and 220) of the MDR1 gene (Accession No: M29445) is associated with low PGP expression-related low drug efflux and patient carrying this substitution are more likely to respond to irinotecan treatment for two reasons: 1) Due to the lower amount of PGP in enterocytes more irinotecan can enter the body across the intestinal barrier causing more irinotecan to reach its site of action, the tumor. 2) Due to the lower amount of PGP in the tumor cell membranes more irinotecan can penetrate into the tumor cells to deploy its cytotoxic effects. The currently used standard dose of irinotecan kills highly effective most tumor cells within the first cycles of chemotherapy with only very few surviving drug-resistant tumor cells and tolerable adverse events. Independently from the mechanisms of drug resistance, in these patients, the number of surviving cells is too small to develop into a drug-resistant tumor which does not respond any longer to irinotecan therapy.

Patients with the high expresser MDR1 genotype (nucleotide C at position 176 of the MDR1 gene, Accession No: M29445) are less likely to respond to irinotecan treatment. Higher doses would be necessary to achieve a sufficiently efficient killing of tumor cells in order to prevent the development of a drug-resistant tumor. However, elevation of irinotecan dosage is limited due to the occurrence of intolerable adverse events (e.g. diarrhea, neutropenia, or thromboembolic complications). Alternatively, efficacy of irinotecan treatment can be improved by

addition of a PGP inhibitor. A PGP inhibitor blocks efficiently the PGP function in MDR1 high expresser patients in such a way as to enable irinotecan to concentrate in the tumor cells for exerting its cytotoxicity as effective as in MDR1 low expresser patients. Consequently, genotypically MDR1 high expresser patients become phenotypically comparable to MDR1 low expressers.

According to the number of low or high expresser alleles of the MDR1 gene, individuals can be classified as having either extensive (ET, two high expresser alleles), intermediate (IT, one high expresser, one low expresser allele) or poor transport capacity (PT, two low expresser alleles). By genetic testing prior to onset of treatment with irinotecan, patients can be classified as ET, IT, or PT and the MDR1-related transport capacity of the patients can be predicted. The individual risk of an insufficient anticancer treatment increases with the number of MDR1 high expresser alleles. Individuals with ET genotype are at the highest risk to suffer from insufficient response to irinotecan and are at the highest risk to develop a drug resistant tumor. ET patients should be treated with a PGP-inhibitor in addition to irinotecan and more closely monitored for adverse events and for the development of chemotherapy-related drug-resistance. Furthermore, these patients, who are at high risk for developing a drug-resistant tumor, can particularly benefit from taking a tumor biopsy between each cycle of chemotherapy with subsequent individual profiling of tumor cells for drug resistance.

## Claims

1. A method of using irinotecan to treat a patient suffering from cancer which comprises:
  - (a) determining if the patient has one or more variant alleles of the UGT1A1 gene;
  - (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.
2. The method of claim 1 wherein the cancer is colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, or pancreatic cancer.
3. The method of claim 2 in which:
  - (a) the one or more variant alleles result in the patient expressing low amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is decreased to avoid toxicity; or
  - (b) the one or more variant alleles result in the patient expressing high amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is increased to enhance efficacy.
4. The method of claim 3 wherein the one or more variant alleles are in the promoter region of the UGT1A1 gene.

5. The method of claim 3 wherein the one or more variant alleles are in the coding region of the UGT1A1 gene.
6. The method of claim 3 wherein the one or more variant alleles are not in either the promoter region or the coding region of the UGT1A1 gene.
7. The method of claim 3 wherein the one or more variant alleles are in both the promoter region and the coding region of the UGT1A1 gene.
8. The method of claim 3 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:
  - (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 001, 002, 005, 006, 009, 010, 013, 014, 017, 018, 021, 022, 025, 026, 029, 030, 033, 034, 037, 038, 041, 042, 045, 046, 049, 050, 053, 054, 057, 058, 061, 062, 065, 066, 069, 070, 073, 074, 077, 078, 081, 082, 085, 086, 089, 090, 093, 094, 097, 098, 101, 102, 105, 106, 109, 110, 113, 114, 129, 130, 133 and/or 134;
  - (b) a polynucleotide encoding a polypeptide having the amino acid sequence of any one of SEQ ID NOs: 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596 and/or 598;
  - (c) a polynucleotide capable of hybridizing to a Uridine Diphosphate Glycosyltransferase1 Member A1 (UGT1A1) gene, wherein said polynucleotide is having at a position corresponding to positions 59, 160, 226, 539, 544, 640, 701, 841, 855, 890, 938, 1006, 1007, 1020, 1084, 1085, 1114, 1117, 1139, 1158, 1175 to 1176, 1216, 1297, 1324, 1471, 1478, 372 to 373, 523 to 525, and/or 892 to 905 of the UGT1A1 gene (Accession No. GI:8850235), a substitution or deletion of at least one nucleotide or at a position corresponding to positions 470/471, and/or

1222/1223 of the UGT1A1 gene (Accession No. GI:8850235) a insertion of at least one nucleotide;

- (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having at a position corresponding to position 226, 539, 701, 855, 938, 1020, and/or 1117 of the UGT1A1 gene (Accession No: GI:8850235) an A, at a position corresponding to position 160, 640, 890, 1006, 1084, 1139, 1176, 1324, and/or 1478 of the UGT1A1 gene (Accession No: GI: 8850235) a T, at a position corresponding to position 544, 841, and/or 1216 of the UGT1A1 gene (Accession No: GI: 8850235) a C, at a position corresponding to position 59, 1007, 1085, 1114, 1158, 1175, 1297, and/or 1471 of the UGT1A1 gene (Accession No: GI:181303) a G, and/or at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TTC, at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TACATTAATGCTTC, at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a T, and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a G;
- (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution of Leu to Arg at a position corresponding to position 15 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 71 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Leu to Gln at a position corresponding to position 175 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Cys to Arg at a position corresponding to position 177 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Trp at a position corresponding to position 209 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Gln at a position corresponding to position 229 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 276 of the UGT1A1

polypeptide (Accession No: G8850236) or/and Ala to Val at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Trp at a position corresponding to position 293 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Glu at a position corresponding to position 308 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding to position 331 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding to position 357 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Gly at a position corresponding to position 367 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Thr at a position corresponding to position 368 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Arg at a position corresponding to position 387 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a position corresponding to position 375 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Arg at a position corresponding to position 381 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Pro at a position corresponding to position 401 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Lys to Glu at a position corresponding to position 428 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Asp at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a position corresponding to position 488 of the UGT1A1 polypeptide (Accession No: G8850236);

- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide is having at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, whereby in said polypeptide one or more aminoacids following amino acid Asp at a position corresponding to position 119 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a T, whereby in said polypeptide one or more aminoacids following amino acid Pro at a position corresponding to position 152 of the

UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TTC, whereby in said polypeptide one or more aminoacids following amino acid Thr at a position corresponding to position 168 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TACATTAATGCTTC, whereby in said polypeptide one or more aminoacids following amino acid Ala at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a G, whereby in said polypeptide one or more aminoacids following amino acid Lys at a position corresponding to position 402 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted; and

- (g) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide comprises an amino acid substitution of Gln to a stop codon at a position corresponding to position 49 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Cys to a stop codon at a position corresponding to position 280 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 331 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Trp to a stop codon at a position corresponding to position 335 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 357 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Lys to a stop codon at a position corresponding to position 437 of the UGT1A1 gene (Accession No: G8850236).



9. The method of claim 8 wherein the one or more variant alleles comprises a polynucleotide selected from the group consisting of:

- (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NO: 37, 69 or 97;
- (b) a polynucleotide encoding a polypeptide having the amino acid sequence of SEQ ID NO: 558, 570 or 584;
- (c) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having a substitution at a position corresponding to position 890, 1117 or 1471 of the UGT1A1 gene (Accession No: GI: 8850235);
- (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having an A at a position corresponding to position 1117, a T at a position corresponding to position 890 or a G at a position corresponding to position 1471 of the UGT1A1 gene (Accession No: GI:8850235);
- (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution at a position corresponding to position 292, 368 or 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236); and
- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises amino acid substitution of Ala to Val at a position corresponding to position 292, Ala to Thr at a position corresponding to position 368 or Tyr to Asp at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: GI: 8850236).

10. The method of claim 8 in which the one or more variant alleles results in the patient expressing low amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is decreased.

11. The method of claim 8 in which the one or more variant alleles results in the patient expressing high amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is increased.
12. The method of claim 9 in which the one or more variant alleles results in the patient expressing low amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is decreased.
13. The method of claim 9 in which the one or more variant alleles results in the patient expressing high amounts of the UGT1A1 gene product, whereby the amount of irinotecan administered to the patient is increased.
14. A method for determining whether a patient is at risk for a toxic reaction to treatment with irinotecan which comprises determining if the patient has one or more variant alleles of the UGT1A1 gene.
15. The method of claim 14 which further comprises administering to the patient reduced amounts of irinotecan if the patient has one or more variant alleles that result in decreased expression of the UGT1A1 gene.
16. A method for determining the optimum treatment regimen for administering irinotecan to a patient suffering from cancer which comprises:
  - (1) determining if the patient has one or more variant alleles of the UGT1A1 gene;
  - (2) in a patient having one or more of such alleles increasing or decreasing the amount of irinotecan in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.

17. A method of treating cancer in a patient having one or more variant alleles of the UGT1A1 gene such that expression levels of the UGT1A1 gene product are lower than in the general population and so indicates high sensitivity to irinotecan which comprises administering to the patient a decreased amount of irinotecan.
18. A method of treating cancer in a patient having one or more variant alleles of the UGT1A1 gene such that expression levels of the UGT1A1 gene product are higher than in the general population and so indicates resistance or predisposition to resistance to irinotecan which comprises administering to the patient an increased amount of irinotecan.
19. The method of claim 18 in which patients that have a variant allele that indicates resistance or predisposition to resistance are treated with an UGT1A1 inhibitor.
20. The method of claim 19 wherein the UGT1A1 inhibitor is selected from the group consisting of:  $\beta$ -estradiol, 4-hydroxyestrone, 2-hydroxyestrone, 7,8-Benzoflavone, Quercetin, Naringenin, Chrysin, Bilirubin, and Octylgallate.
21. The method of claim 17 which further comprises monitoring the patient during treatment by assaying for changes in expression levels of the UGT1A1 gene product in the cancerous cells whereby an increase in the expression level of the UGT1A1 gene product is compensated for by an increase in the amount of irinotecan administered to the patient.
22. A method of treating cancer in a patient which comprises internally administering to the patient an effective amount of irinotecan, wherein the treatment regimen is modified based upon the genotype of the patient's UGT1A1 gene.

23. A method of treating a population of patients suffering from cancer which comprises:
- (a) determining, on a patient by patient basis, if the patient has one or more variant alleles of the UGT1A1 gene;
  - (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which is sufficient to treat a patient having such variant alleles which amount is increased or decreased in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.
24. A method of using irinotecan to treat a patient having Gilbert Syndrome who is suffering from cancer which comprises:
- (a) determining if the patient has one or more variant alleles of the UGT1A1 gene which results in low production or glucuronidation activity of the corresponding protein;
  - (b) in a patient having one or more of such variant alleles, administering to the patient an amount of irinotecan which amount is decreased in comparison to the amount that is administered without regard to the patient's alleles in the UGT1A1 gene.
25. A method of treating cancer in a patient having Gilbert Syndrome which comprises internally administering to the patient an effective amount of irinotecan, wherein the treatment regimen is modified based upon the genotype of the patient's UGT1A1 gene.
26. A method for predicting sensitivity to irinotecan in a patient suffering from cancer which comprises determining if the patient has one or more variant alleles of the UGT1A1 gene, which alleles indicate that the cancerous cells express low or high amounts of the UGT1A1 protein, whereby low expression indicates high sensitivity to irinotecan and high expression indicates resistance or predisposition to resistance to irinotecan.

26. A method for predicting sensitivity to irinotecan in a patient suffering from cancer which comprises determining if the patient has one or more variant alleles of the UGT1A1 gene, which alleles indicate that the cancerous cells express low or high amounts of the UGT1A1 protein, whereby low expression indicates high sensitivity to irinotecan and high expression indicates resistance or predisposition to resistance to irinotecan.
- ~~27. The method of claim 26 in which patients that have a genotype that indicates~~  
resistance or predisposition to resistance are treated with a UGT1A1 inhibitor.
28. The method of claim 27 wherein the UGT1A1 inhibitor is selected from the group consisting of:  $\beta$ -estradiol, 4-hydroxyestrone, 2-hydroxyestrone, 7,8-Benzoflavone, Quercetin, Naringenin, Chrysin, Bilirubin, and Octylgallate.
29. The method of claim 26 wherein the patients that have a genotype that indicates resistance or predisposition to resistance are monitored during treatment by assaying for expression levels of the UGT1A1 gene product in the cancerous cells.
30. Use of irinotecan or a derivative thereof for the preparation of a pharmaceutical composition for treating or preventing colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer in a subject having a genome with a first variant allele which comprises a polynucleotide selected from the group consisting of:
- (a) a polynucleotide having the nucleic acid sequence of any one of SEQ ID NOs: 001, 002, 005, 006, 009, 010, 013, 014, 017, 018, 021, 022, 025, 026, 029, 030, 033, 034, 037, 038, 041, 042, 045, 046, 049, 050, 053, 054, 057, 058, 061, 062, 065, 066, 069, 070, 073, 074, 077, 078, 081, 082, 085, 086, 089, 090, 093, 094, 097, 098, 101, 102, 105, 106, 109, 110, 113, 114, 129, 130, 133 and/or 134;

- (b) a polynucleotide encoding a polypeptide having the amino acid sequence of any one of SEQ ID NOs: 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596 and/or 598;
- (c) a polynucleotide capable of hybridizing to a Uridine Diphosphate Glycosyltransferase1 Member A1 (UGT1A1) gene, wherein said polynucleotide is having at a position corresponding to positions 59, 160, ~~226, 539, 544, 640, 701, 841, 855, 890, 938, 1006, 1007, 1020, 1084,~~ 1085, 1114, 1117, 1139, 1158, 1175 to 1176, 1216, 1297, 1324, 1471, 1478, 372 to 373, 523 to 525, and/or 892 to 905 of the UGT1A1 gene (Accession No. GI:8850235), a substitution or deletion of at least one nucleotide or at a position corresponding to positions 470/471, and/or 1222/1223 of the UGT1A1 gene (Accession No. GI:8850235) a insertion of at least one nucleotide;
- (d) a polynucleotide capable of hybridizing to a UGT1A1 gene, wherein said polynucleotide is having at a position corresponding to position 226, 539, 701, 855, 938, 1020, and/or 1117 of the UGT1A1 gene (Accession No: GI:8850235) an A, at a position corresponding to position 166, 640, 890, 1006, 1084, 1139, 1176, 1324, and/or 1478 of the UGT1A1 gene (Accession No: GI: 8850235) a T, at a position corresponding to position 544, 841, and/or 1216 of the UGT1A1 gene (Accession No: GI: 8850235) a C, at a position corresponding to position 59, 1007, 1085, 1114, 1158, 1175, 1297, and/or 1471 of the UGT1A1 gene (Accession No: GI:181303) a G, and/or at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TTC, at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of TACATTAATGCTTC, at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a T, and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850235) a insertion of a G;a polynucleotide capable of hybridizing to a Uridine Diphosphate Glycosyltransferase1 Member A1

(UGT1A1) gene, wherein said polynucleotide is having at a position corresponding to positions 58, 166, 226, 539, 544, 640, 701, 841, 855, 890, 938, 1006, 1007, 1020, 1084, 1085, 1114, 1117, 1139, 1158, 1175 to 1176, 1216, 1297, 1324, 1471, 1488, 372 to 373, 523 to 525, and/or 892 to 905 of the UGT1A1 gene (Accession No. GI:8850235), a substitution or deletion of at least one nucleotide or at a position corresponding to positions 470/471, and/or 1222/1223 of the UGT1A1 gene (Accession No. GI:8850235) a insertion of at least one nucleotide;

- (e) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution at a position corresponding to positions 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, and/or 586 of the UGT1A1 polypeptide (Accession No: G8850236);
- (f) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polypeptide comprises an amino acid substitution of Leu to Arg at a position corresponding to position 15 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 71 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Leu to Gln at a position corresponding to position 175 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Cys to Arg at a position corresponding to position 177 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Trp at a position corresponding to position 209 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Gln at a position corresponding to position 229 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Arg at a position corresponding to position 276 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Val at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Trp at a position corresponding to position 293 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gly to Glu at a position corresponding to position 308 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding

to position 331 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Gln to Arg at a position corresponding to position 357 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Arg to Gly at a position corresponding to position 367 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Thr at a position corresponding to position 368 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Pro to Arg at a position corresponding to position 387 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a position corresponding to position 375 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Arg at a position corresponding to position 381 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ala to Pro at a position corresponding to position 401 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Lys to Glu at a position corresponding to position 428 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Tyr to Asp at a position corresponding to position 486 of the UGT1A1 polypeptide (Accession No: G8850236) or/and Ser to Phe at a position corresponding to position 488 of the UGT1A1 polypeptide (Accession No: G8850236);

- (g) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide is having at a position corresponding to position 372 to 373 of the UGT1A1 gene (Accession No: GI:8850235) a deletion of CT, whereby in said polypeptide one or more aminoacids following amino acid Asp at a position corresponding to position 119 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 470/471 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a T, whereby in said polypeptide one or more aminoacids following amino acid Pro at a position corresponding to position 152 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 523 to 525 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TTC, whereby in said polypeptide one or more aminoacids following amino acid Thr at a position corresponding to position 168 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or



at a position corresponding to position 892 to 905 of the UGT1A1 gene (Accession No: GI:8850236) a deletion of TACATTAATGCTTC, whereby in said polypeptide one or more aminoacids following amino acid Ala at a position corresponding to position 292 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted and/or at a position corresponding to position 1222/1223 of the UGT1A1 gene (Accession No: GI:8850236) a insertion of a G, whereby in said polypeptide one or more aminoacids following amino acid Lys at a position corresponding to position 402 of the UGT1A1 polypeptide (Accession No: G8850236) are substituted, added, and/or deleted; and

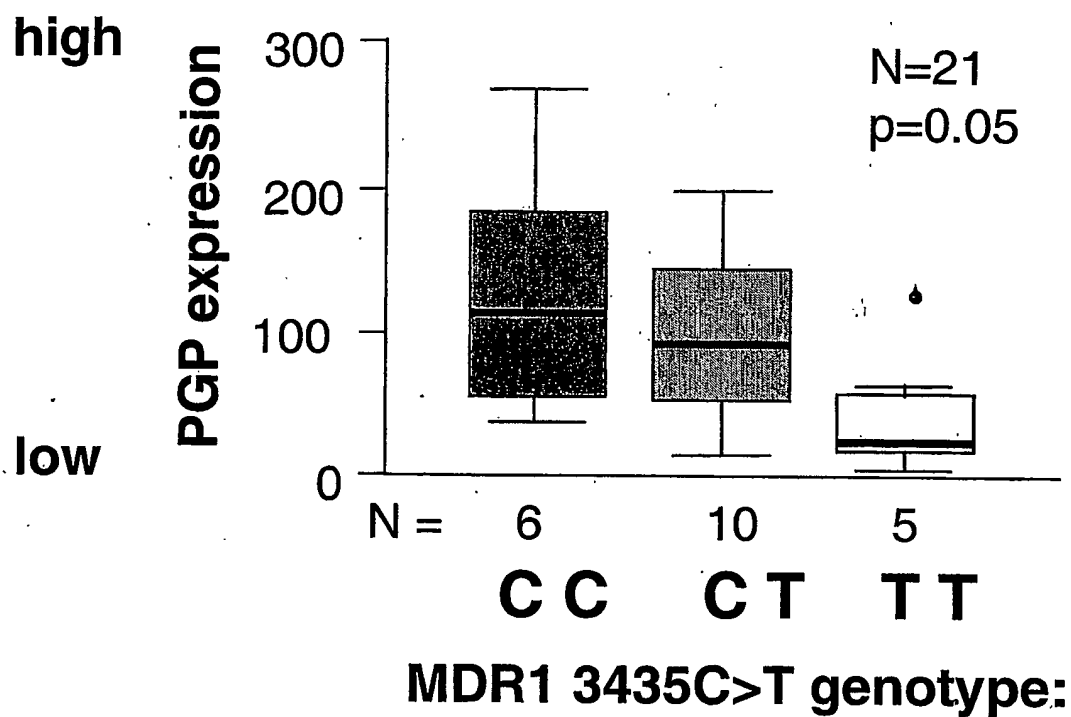
- (h) a polynucleotide encoding an UGT1A1 polypeptide or fragment thereof, wherein said polynucleotide comprises an amino acid substitution of Gln to a stop codon at a position corresponding to position 49 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Cys to a stop codon at a position corresponding to position 280 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 331 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Trp to a stop codon at a position corresponding to position 335 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Gln to a stop codon at a position corresponding to position 357 of the UGT1A1 gene (Accession No: G8850236) and/or an amino acid substitution of Lys to a stop codon at a position corresponding to position 437 of the UGT1A1 gene (Accession No: G8850236).

31. The use of claim 30, wherein a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered expression of the variant allele compared to the corresponding wild type alleles.

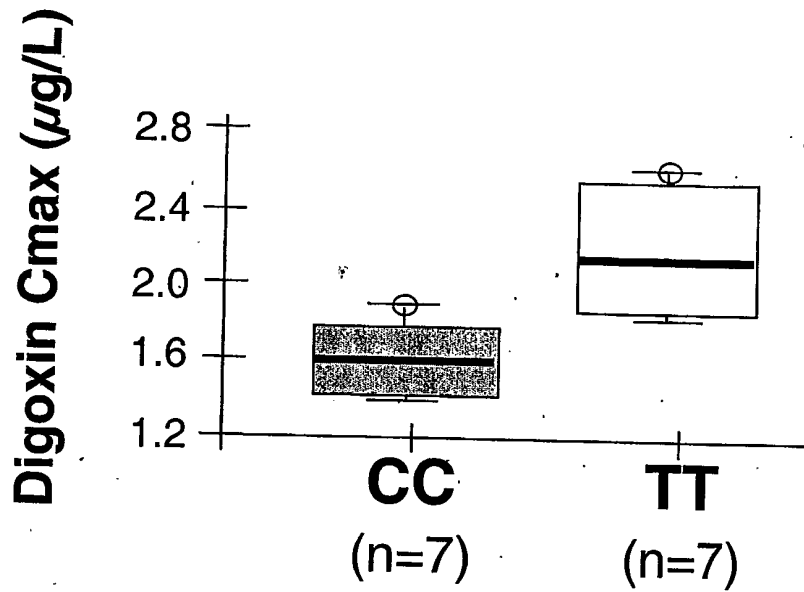
32. The use of claim 31, wherein said altered expression is decreased or increased expression.

33. The use of claim 30, wherein a nucleotide deletion, addition and/or substitution comprised by said polynucleotide results in an altered activity of the polypeptide encoded by the variant allele compared to the polypeptide encoded by the corresponding wild type allele.
34. The use of claim 33, wherein said altered activity is decreased or increased activity.
35. The use of any one of claims 30 to 34, wherein said subject is an animal.
36. The use of claim 35, wherein said subject is a mouse.
37. The use of any one of claims 30 to 34, wherein said subject is a human.
38. The use of claim 37, wherein said human is African or Asian.
39. A method for selecting a suitable therapy for a subject suffering from colorectal cancer, cervical cancer, gastric cancer, lung cancer, malignant glioma, ovarian cancer, and pancreatic cancer, wherein said method comprises:
- (a) determining the presence or absence of a variant allele as specified in claim 30 in the genome of a subject in a sample obtained from said subject; and
  - (b) selecting a suitable therapy for said subject based on the results obtained in (a).

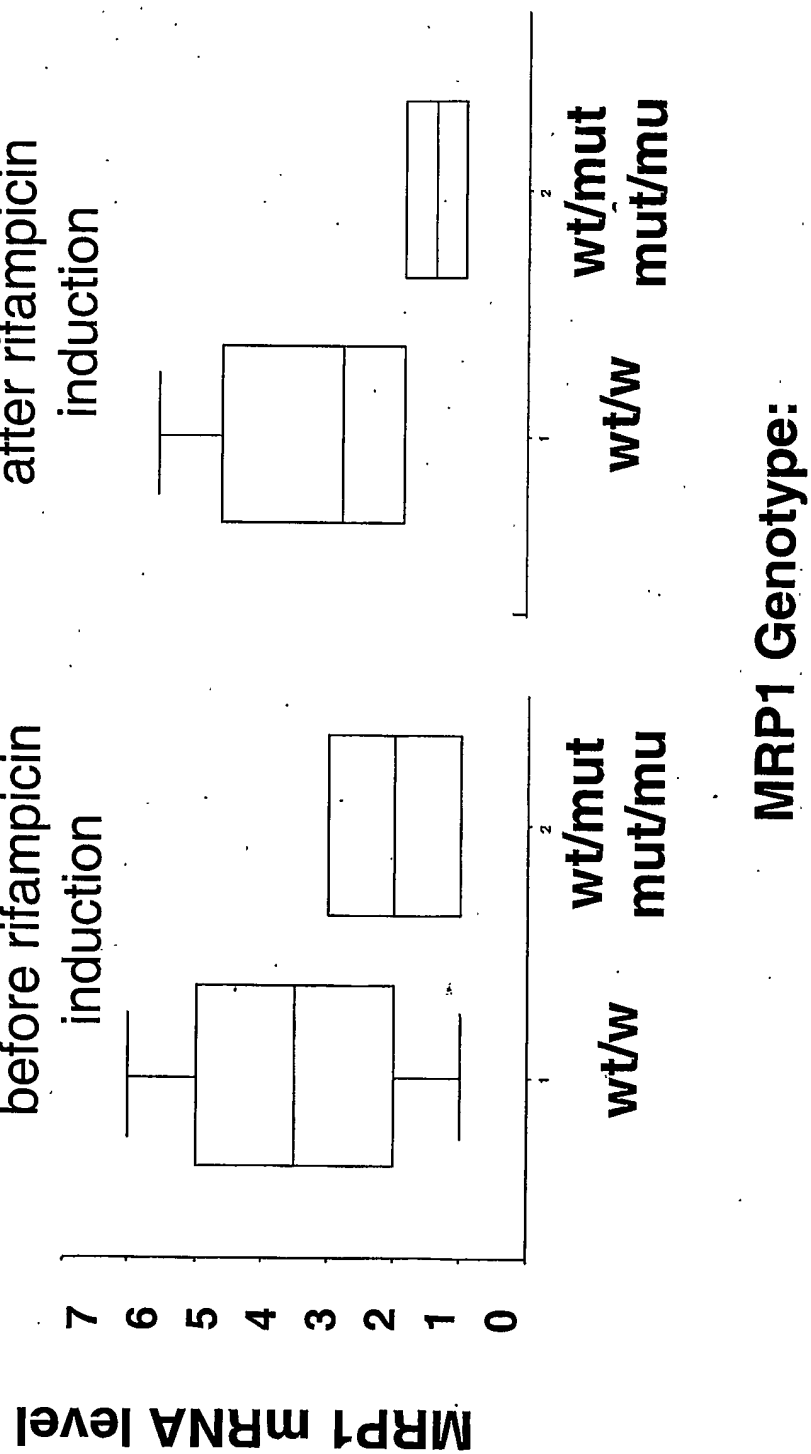
1/1401

**Figure 1**

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**Figure 2****MDR1 3435C>T genotype:**

**Figure 3**

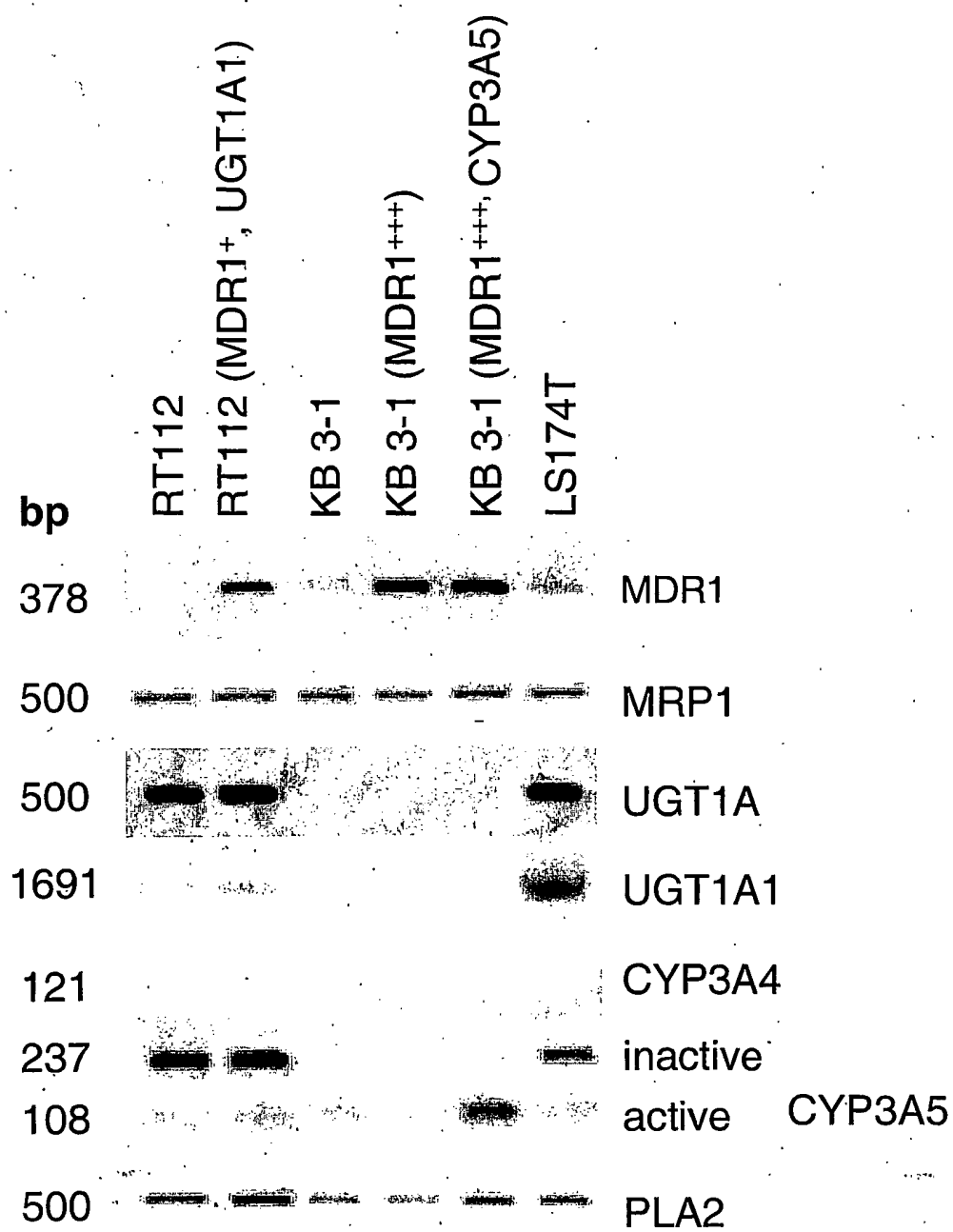


**Figures 4 to 28**

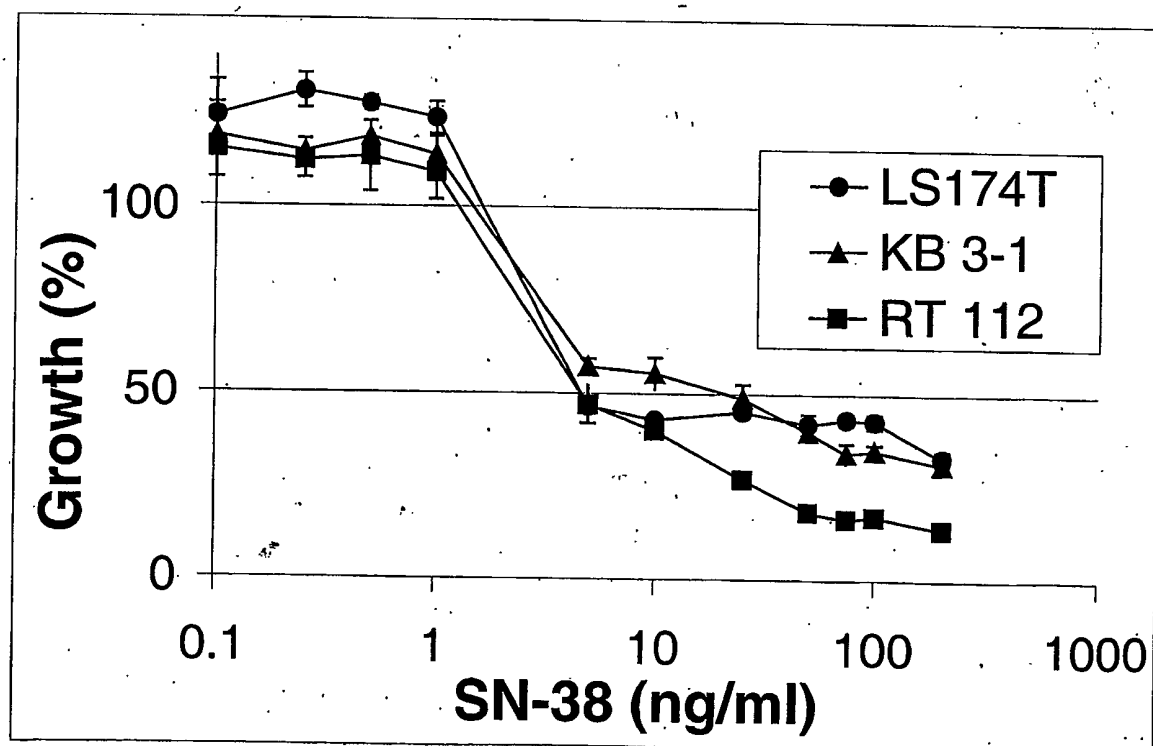
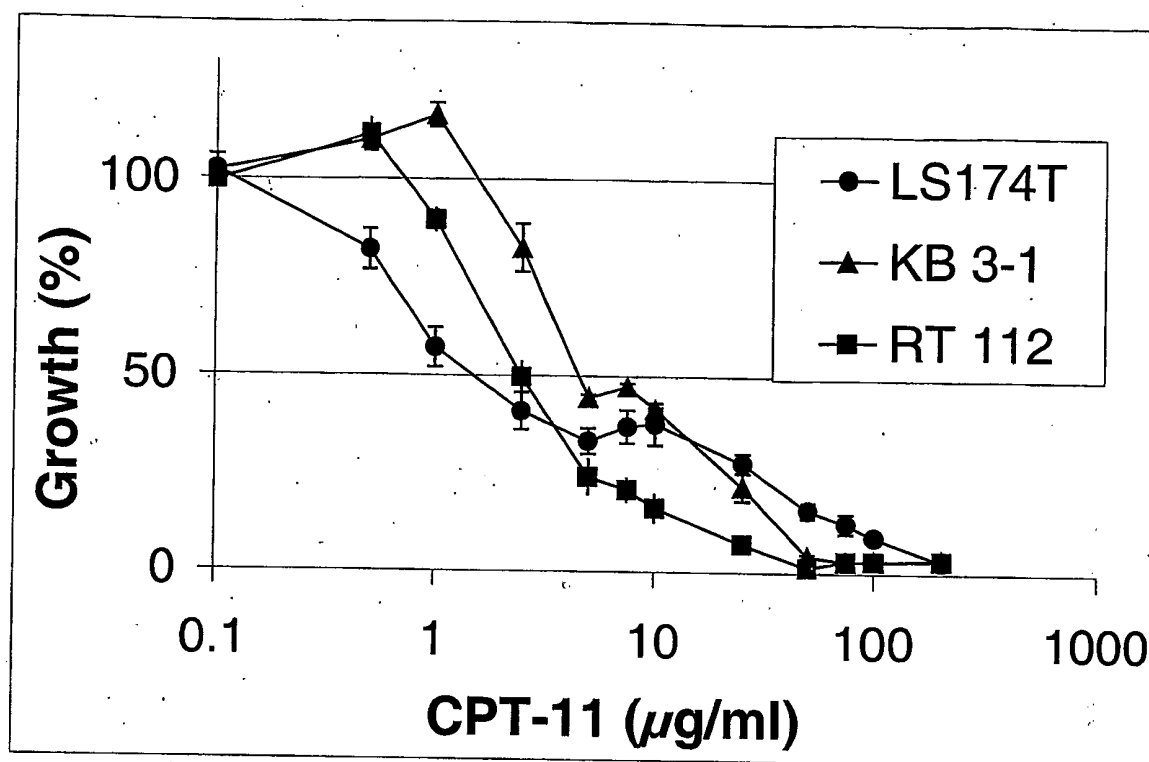
represent sequence listings published separately in electronic form and are available upon request from the International Bureau or can be viewed from the following WIPO website :

<http://www.wipo.int/pct/en/sequences/index.htm>

1396/1401

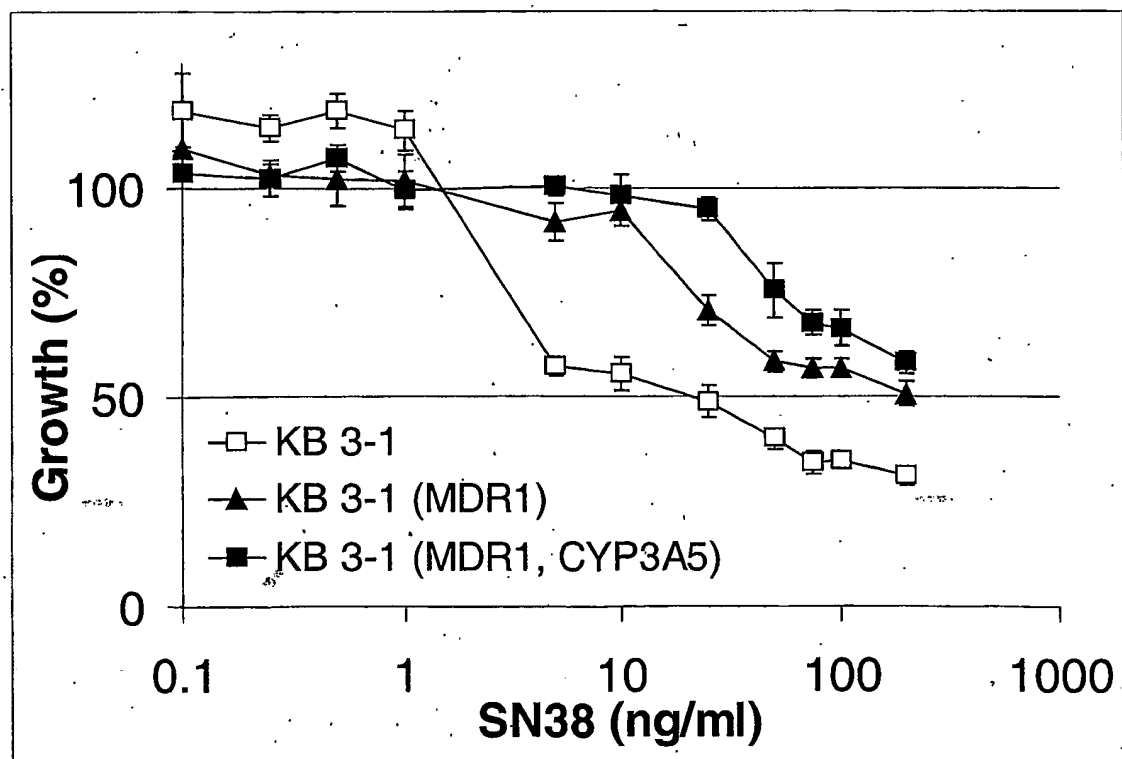
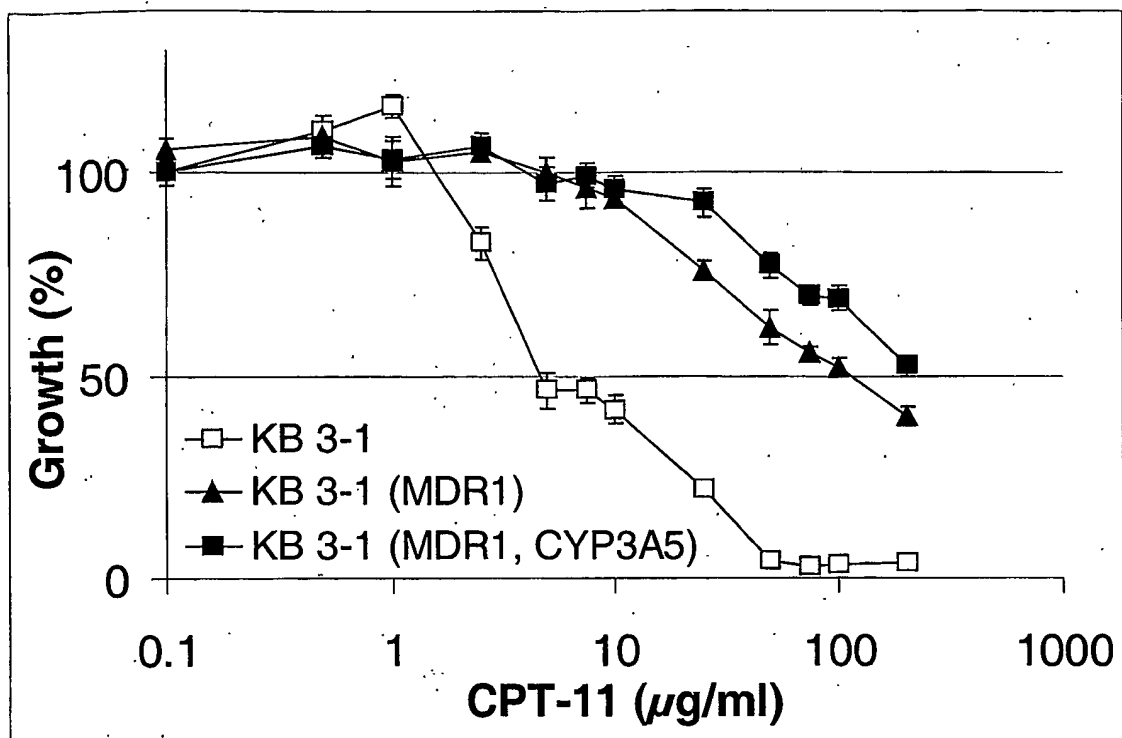
**Figure 29**

1397/1401

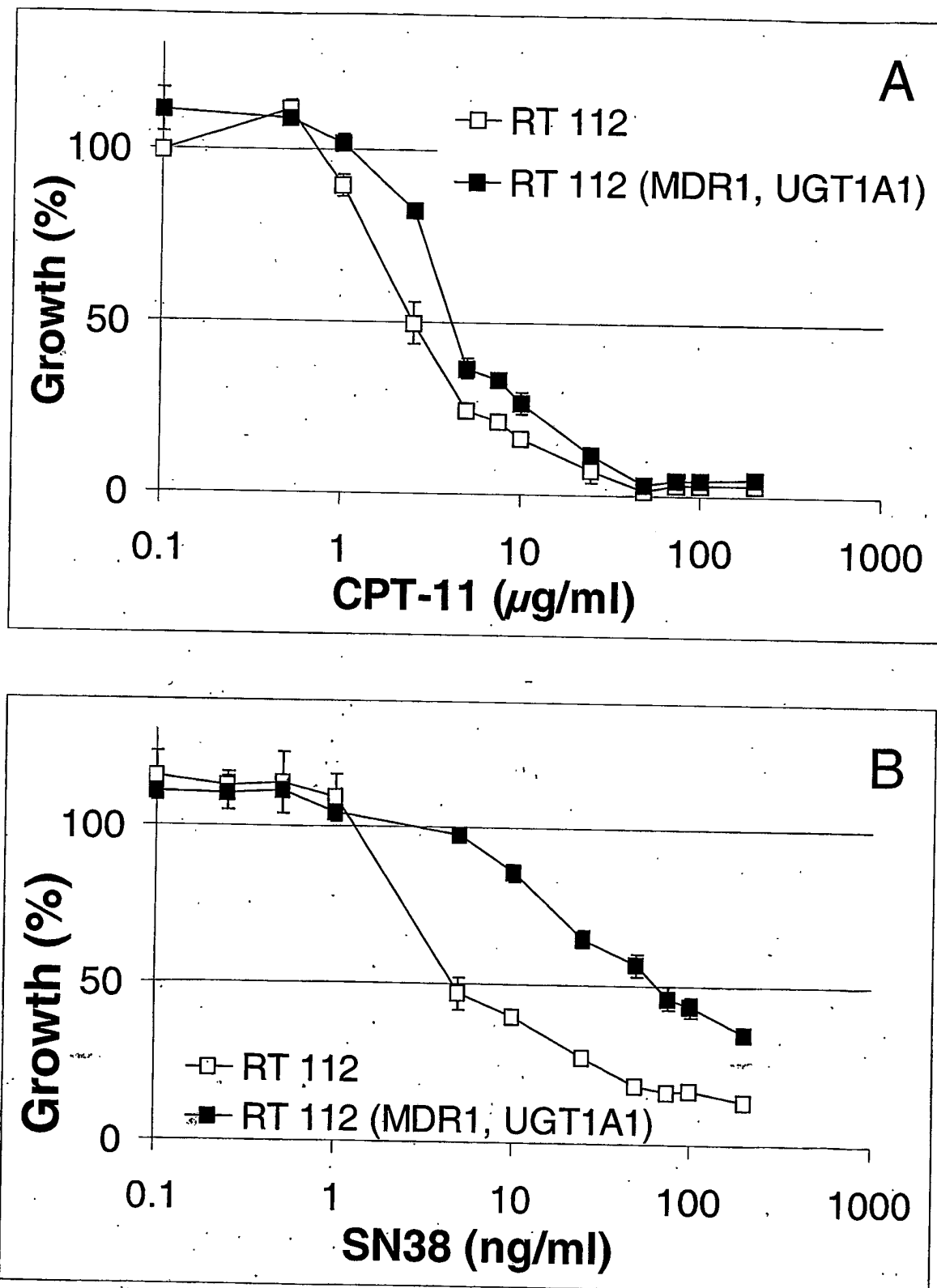
**Figure 30**



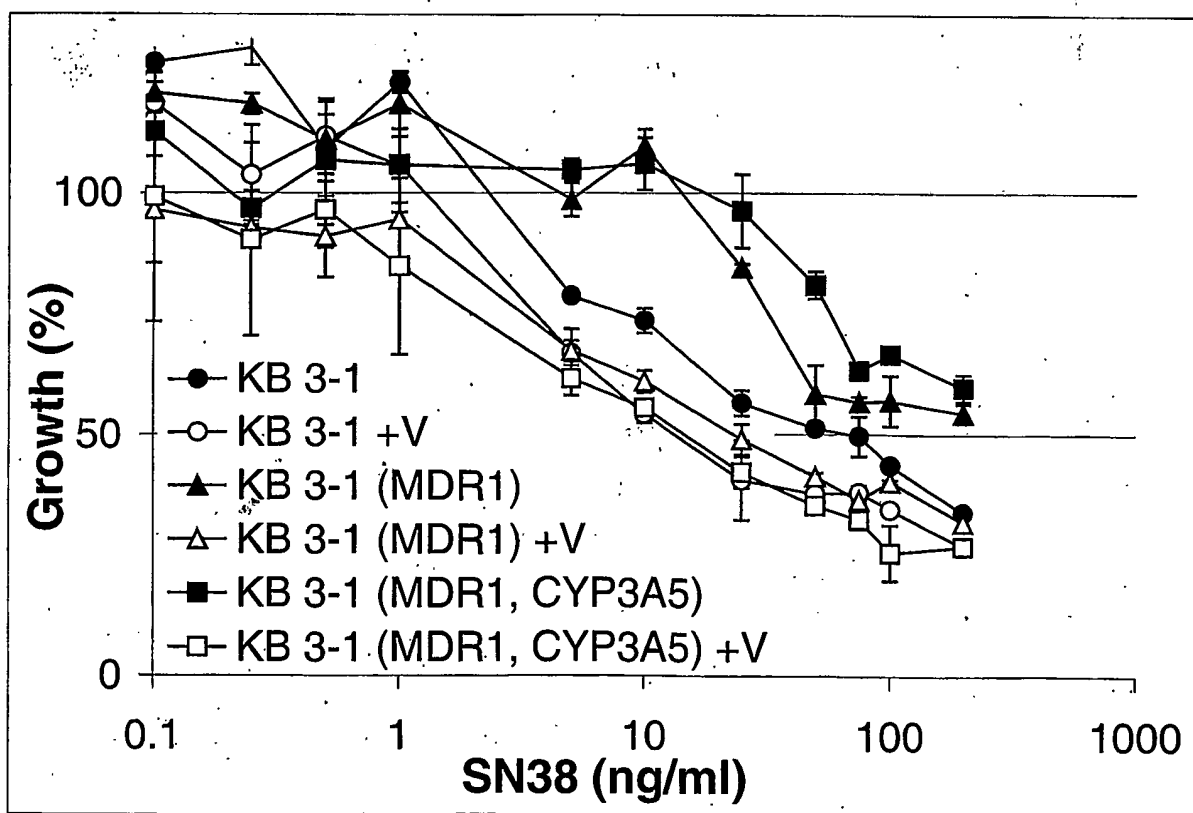
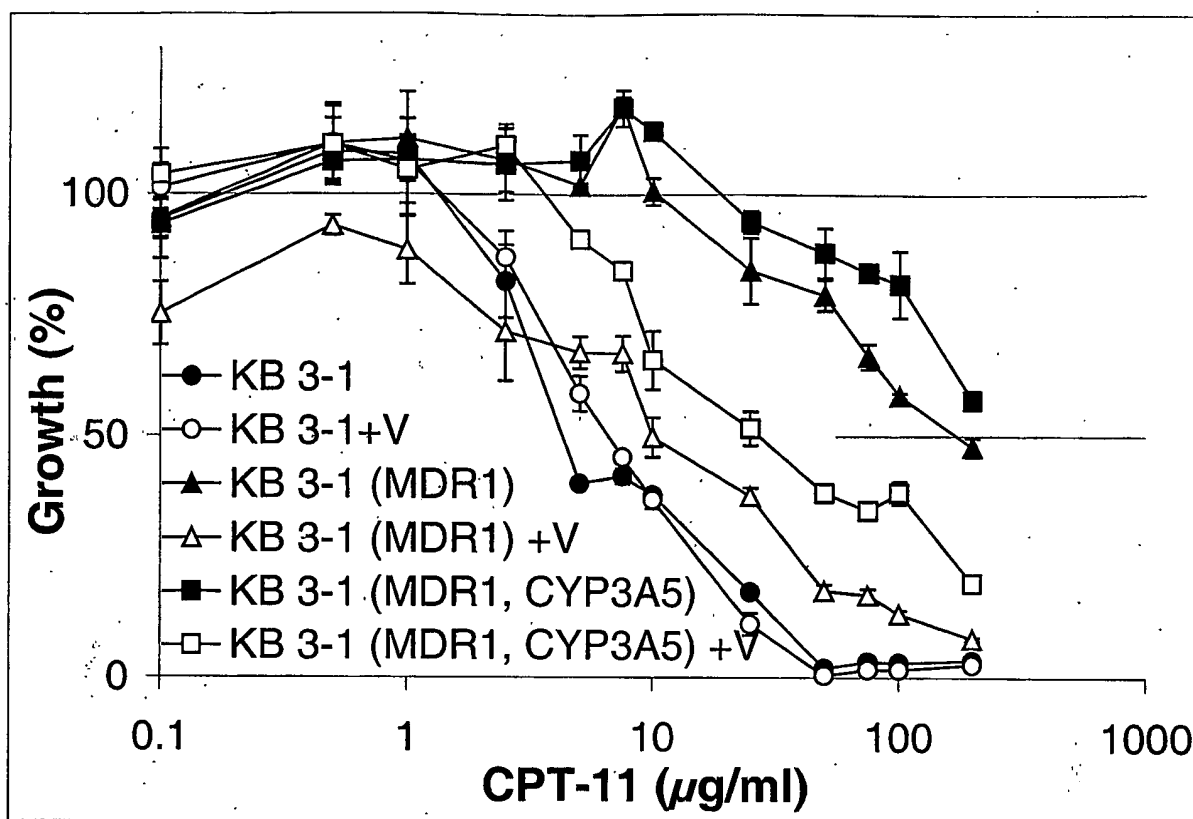
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**Figure 31**

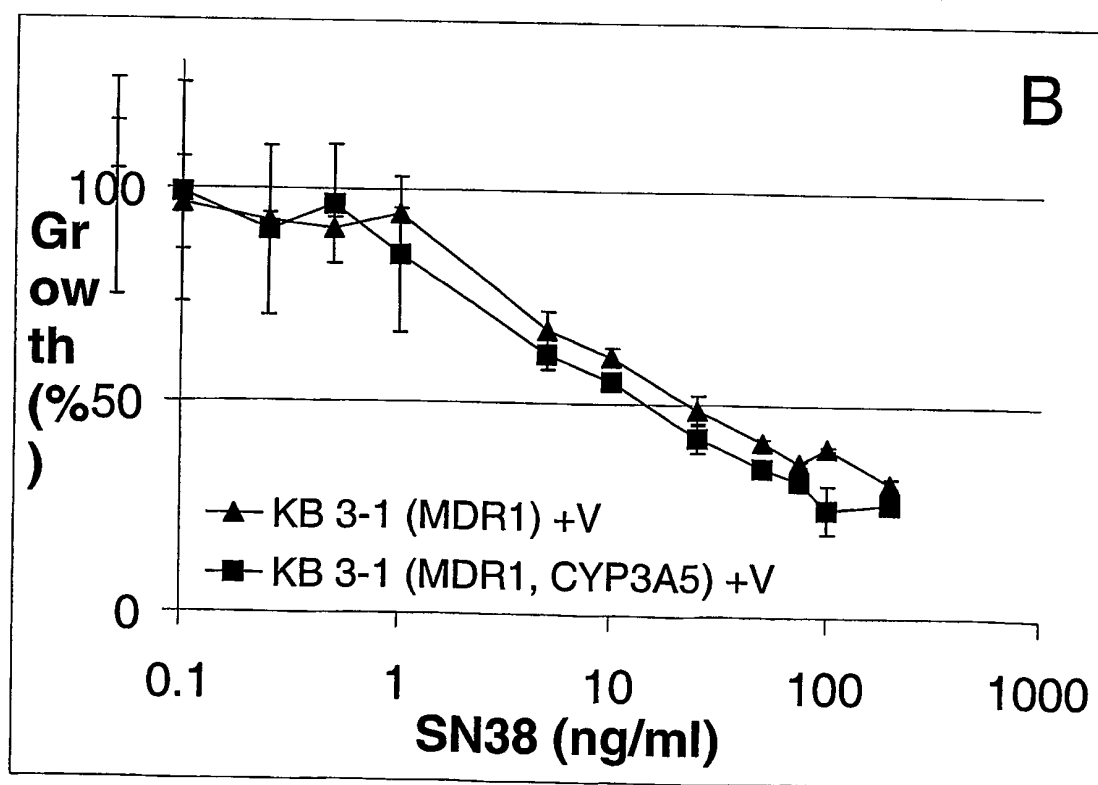
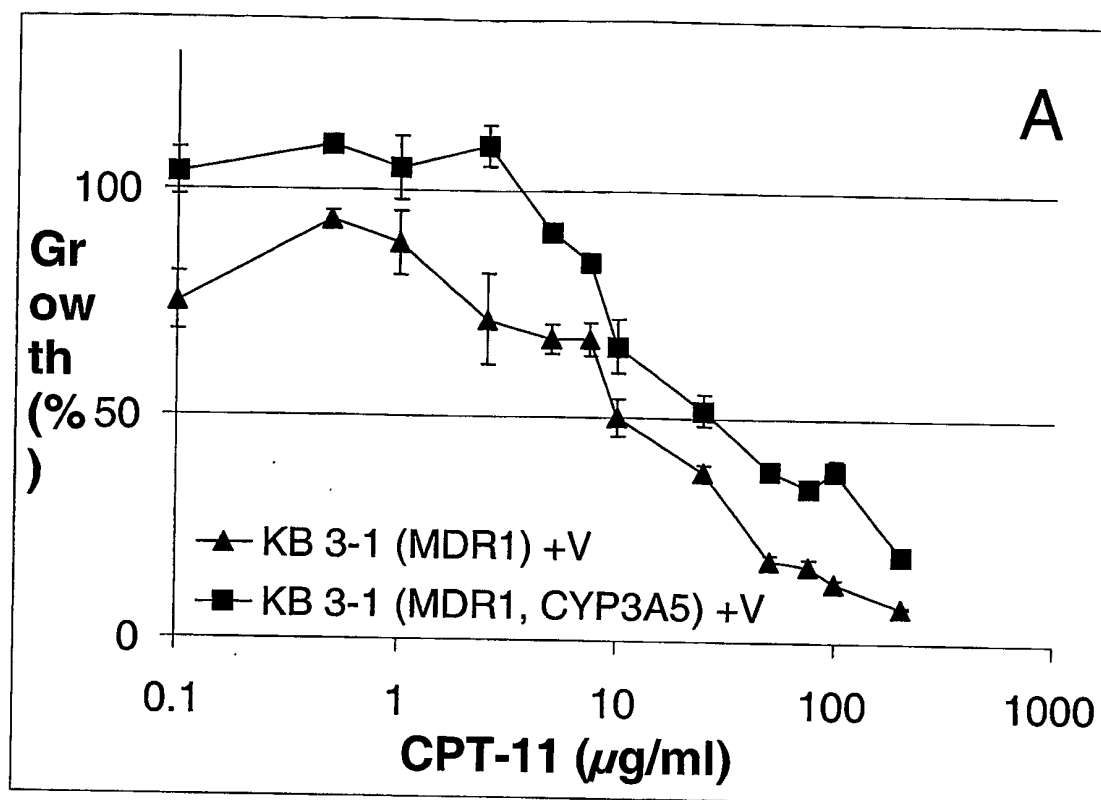
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**Figure 32**

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**Figure 33**

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**Figure 34**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/08217

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K31/4745 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 37892 A (UPJOHN CO ;ULRICH ROGER G (US)) 3 September 1998 (1998-09-03) page 1, line 5 - line 26	19-21, 27-29
X	WO 00 37107 A (MASFERRER JAIME L ;GORDON GARY (US); SEARLE & CO (US); KOKI ALANE) 29 June 2000 (2000-06-29) claims 1,14,42	1-18,22, 23,26, 30-39 19-21, 27-29
A	WO 01 49299 A (MICHAEL MICHAEL ;MOORE MALCOLM J (CA)) 12 July 2001 (2001-07-12) page 2, line 1 - line 22 page 3, line 5 - line 21	1-18,22, 23,26, 30-39 14-16
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

26 June 2003

Date of mailing of the international search report

16. 10. 2003

Name and mailing address of the ISA

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Authorized officer

Bonzano, C.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/08217

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 54678 A (SCHERING CORP) 2 August 2001 (2001-08-02)  page 1, line 16 - line 19 page 3, line 11 - line 19 -----	1-13,17, 18,22, 23,30-39
P,X	WO 02 28380 A (MARTIN CHRISTOPHE ;BAILEY SUSAN M (GB); TWELVES CHRISTOPHE J (GB);) 11 April 2002 (2002-04-11) page 1, line 16 - line 24 claims 1,8 -----	1-18,22, 23,26, 30-39
P,X	WO 01 87306 A (TRUSTEES OF THE UNIVERSITY OF ;CELGENE CORP (US)) 22 November 2001 (2001-11-22) example 1 -----	1-18,22, 23,26, 30-39
X	FRIEDMAN HENRY S ET AL: "Irinotecan therapy in adults with recurrent or progressive malignant glioma." JOURNAL OF CLINICAL ONCOLOGY, vol. 17, no. 5, May 1999 (1999-05), pages 1516-1525, XP008018482 ISSN: 0732-183X the whole document -----	1-18,22, 23,26, 30-39
X	INNOCENTI F ET AL: "PHARMACOGENETICS OF ANTICANCER AGENTS: LESSONS FROM AMONAFIDE AND IRINOTECAN" DRUG METABOLISM AND DISPOSITION, WILLIAMS AND WILKINS., BALTIMORE, MD, US, vol. 29, no. 4, PART 2, April 2001 (2001-04), pages 596-600, XP001008560 ISSN: 0090-9556 page 597, column 2, paragraph 3 - paragraph [0004] page 598, column 1, paragraph 3 page 598, column 2, paragraph 1 - paragraph [0004] page 599, column 1, paragraph 3 -----	14-16, 26-29,39
X	ANDO Y ET AL: "POLYMORPHISMS OF UDP-GLUCURONOSYLTRANSFERASE GENE AND IRINOTECAN TOXICITY: A PHARMACOGENETIC ANALYSIS" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 60, 15 December 2000 (2000-12-15), pages 6921-6926, XP002909302 ISSN: 0008-5472 -----	1-18,22, 23,26, 30-39
A	page 6922, column 1, paragraph 1  page 6925, column 2, paragraph 2 - paragraph [0003] -----	14-16, 26,27,37
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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 02/08217

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>IYER L ET AL: "GENETIC PREDISPOSITION TO THE METABOLISM OF IRINOTECAN (CPT-11) ROLE OF URIDINE DIPHOSPHATE GLUCURONOSYLTRANSFERASE ISOFORM 1A1 IN THE GLUCURONIDATION OF ITS ACTIVE METABOLITE (SN-38) IN HUMAN LIVER MICROSOMES" JOURNAL OF CLINICAL INVESTIGATION, NEW YORK, NY, US, vol. 101, no. 4, 15 February 1998 (1998-02-15), pages 847-854, XP001120443 ISSN: 0021-9738 page 852, column 2, paragraph 4 -----</p>	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 02/08217

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-23, 26-39

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.1

Although claims 1-13, 18-25, 30-38 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claims 14-16, 26-29, 39 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-23,26-39

Use of irinotecan for treating colorectal, cevical, gastric, lung, ovarian, pancreatic cancer and malignant glioma. Alone or together with UGT1A1 inhibitors such as in the list of claim 20. Mechanism: UGT1A1 gene is involved. And method of determing the therapy via determining if the patient has the alleles necessary.

---

2. claims: 24,25

Use of irinotecan for treating Gilbert syndrome in cancer patients. Alone or together with AGT1A1 inhibitors such as in the long list of claim 20. Mechanism: AGT1A1 gene. And method of determing the therapy via determining if the patient has the alleles necessary.

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## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 02/08217

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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International Application No

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